THE ACTION OF GASTRIN II ON GASTRIC SECRETION IN MAN

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by

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C O N T E N T S.

CHAPTER X.

A Quantitative Statement of the Two-Component Hypothesis of Gastric Secretion.

CHAPTER 1

INTRODUCTION

A number of authoritative monographs[1,2] have appeared in recent years describing the steps that led to the discovery of the antral hormone, gastrin, and the elucidation of its role in gastric secretion. So that only a brief account will be given here in order to situate the present studies in the main current of renewed interest in this field. For there can be little doubt that the availability of the pure peptides, gastrins I and II (Gregory and Tracy, 1964)[3] and, more recently, of a series of synthetic peptides structurally related to gastrin (Gregory and Tracy, 1964)[4], must lead to a re-examination of the processes of stimulation and inhibition of gastric secretion in man and animal and a re-appraisal of the theoretical considerations on which much of the present knowledge is based.

The Extraction of Gastrin:

The gastrin story appropriately begins with Edkins'[5,6] demonstration that pyloric mucosal extracts contained a "stimulant" of gastric secretion. It was not until a few years later that an attempt was made to delineate the role of the antrum in the secretory response of the stomach[7]. These two parallel attempts at solving the problem are the prototype of much of the work that was to follow. For while many workers directed their efforts at extracting a pyloric substance that could safely be described as a stimulatory/

hormone, others devoted themselves to investigating the role of the antral region in its release.

Much of the controversy that punctuated early research in this field appears, in retrospect, to have been irrelevant. The first damaging blow at the gastrin theory was struck when it was demonstrated [8] that other tissue extracts besides antral extracts contained a powerful stimulant of gastric secretion. By 1919, it had become clear that the stimulant in these extracts was histamine [9,10,11] which in view of its ubiquitous distribution in the body could hardly qualify for the role of a gastric hormone. Sacks et al. [12], who in 1932 made a further attempt at extracting the antral hormone, only succeeded in isolating histamine. Their verdict was suitably equivocal: "either histamine is the gastric hormone, or if not there is no gastric hormone, or the gastric hormone has never been extracted from the pyloric mucosa."

Similar studies [13] around this time showed the absence of histaminase in gastric mucosa and tended to lend support to the conclusion that gastric mucosal histamine might on release play the role of a local or circulating hormone. But other studies describing the mainly fundic distribution of mucosal histamine only served to feed the lingering doubt as to its immediate hormonal role.

In all attempts to isolate gastrin and distinguish it from histamine, one important possibility had been overlooked, namely that the hormone might be a protein, and if so, all the extractive/

extractive procedures in use until then would have removed the hormone and spared the histamine. The possibility that the antral hormone, by analogy with "secretin", was protein-like was first recognised by Komarov[15, 16, 17], working in Babkin's laboratory. Komarov showed that trichloracetic acid could precipitate from simple acid extracts of antral mucosa, a histamine-free, non-toxic protein fraction which elicited, on intravenous administration to conscious dogs or anaesthetised cats, a definite secretory response. He also reported that a certain amount of gastrin-like activity could be found in duodenal extracts, but none in cardiac or fundic extracts. Basing himself on Komarov's findings, Babkin[18] concluded at the time that "the active principle extracted from the mucous membrane of the pyloric part may provisionally be called 'gastrin' with no implication that it plays any part in normal gastric function." and further, "we are not yet entitled to claim that the substance extracted by Komarov from the pyloric mucosa is actually the hormone of this phase, for we still do not understand the mechanism by which the hormone is liberated during gastric digestion...." Although a number of workers[19,20] over the next ten years found it difficult to obtain active preparations of gastrin, others[21,22] using a variety of procedures amply confirmed the findings of Komarov by reporting the consistent presence of gastrin-like activity in antral extracts. The stage was set for the final and most rewarding attempt by Gregory and Tracy[3] at isolating gastrin in pure form from hog antral mucosa.

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The Physiological Role of Gastrin:

Up till the time of Komarov's work, the existence of the antral hormone had not been established by physiological experiments. In the thirty years that followed Edkins' first report, workers in this field could be divided into those who accepted uncritically the existence of a gastric hormonal mechanism, and those who, unconvinced by the evidence to-date, considered it to be a seductive but improbable invention.

The lack of recognition of two important facts had barred the way to any further advance in this direction: (i) that vagal innervation of the antrum and fundus was important for the effective release and action of the antral hormone, and (ii) that release of the hormone could only be effected in the presence of low acidity in the antral region.

The extensive study of Lim, Ivy and McCarthy [23] in 1925 demonstrated that the secretory response to mechanical and chemical stimulation was largely dependent on the presence of the antrum. These workers were prevented from interpreting their results correctly by the earlier failure of Ivy and Whitlow [24] to elicit a response from Pavlov pouches by antral pouch irrigation. Soon after, however, Ivy and Farrell [25] using a transplanted canine fundic pouch showed the presence of a circulating humoral agent following a meal.

Fifteen years elapsed before the search was resumed. In

1941/

1941, Gregory and Ivy [26] re-opened the whole question of the humoral mediation of response. But although they were unsuccessful in locating the site of origin of the humoral agent, they brought forth sufficient evidence to show that it was hormonal in nature and not a secretagogue derived from the products of digestion. Final confirmation, however, came a few years later when it was shown successively that local mechanical stimulation by antral distension [27] and chemical stimulation by antral irrigation with acetylcholine [28] elicited a fundic secretory response.

A number of clues latent in many of these experiments pointed to the possibility that nervous mechanisms were involved in the release of the antral hormone. The early work of Uvnas [29] which because of war conditions had passed unnoticed, contained in germ what has since come to be accepted as one of the major advances in recent years, namely, the recognition of the integration of nervous and hormonal mechanisms in the gastrointestinal tract. It was soon shown on all sides and in numerous experiments, that vagal excitation [30,31] could cause the release of gastrin from an antral mucosa protected from exposure to acid. It has since become clear that on eating a meal, the combined influence of central vagal excitation of the antrum and its local stimulation by mechanical and possibly chemical means causes an immediate release of gastrin. The extent to which the subsequent rise in acidity controls the continued release of the hormone requires further study.

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The fact that concurrent cholinergic stimulation influences the action of gastrin as distinct from its release is now well established. The idea of a synergistic effect between gastrin and cholinergic agents is highly attractive. But considerable doubt persists as to the mechanism by which this effect is mediated. It is not yet clearly established whether this type of co-operation takes the form of a true pharmacological potentiation [32], or is simply coincident on the increase in mucosal blood flow and consequent increase in the delivery of the stimulant to the active sites of secretion [33, 34]. It will be recalled in this context, that the maximal secretory response to histamine is unchanged by vagotomy, although the dose required to elicit this response is much greater after denervation [35]. Whereas a synergistic effect can be demonstrated at the lower dose levels between gastrin and cholinergic agents the maximal secretory response of the intact human stomach remains unaltered (vide infra).

Isolation and Characterisation of Antral Gastrin

The recent report by Gregory and Tracy [3] announcing the isolation of two almost identical peptides, gastrins I and II, is the culmination of many years work on the detection, and physiological study of the active antral principle. The method of extraction described by these workers [36] in 1961 had already made available for the first time a preparation which was highly active on subcutaneous injection to conscious dogs and sufficiently pure for/

for administration to human subjects.

The pure peptides are on a molar basis some 500 times more effective than histamine in stimulating gastric acid secretion. They also possess a duplex effect, which has other pharmacological parallels, in being stimulatory at low doses and inhibitory on rapid delivery in high doses. Several actions shown by the crude antral extracts [37] have been reproduced by the administration of the pure peptides. These include stimulation of gastric and intestinal motility and tone, stimulation of pancreatic volume flow and enzyme output, and a minimal effect on gall-bladder tone and biliary flow. The importance of these findings is in their possible physiological significance; whether, that is, in the intact animal, post-prandial liberation of gastrin not only stimulates gastric acid and pepsin secretion, but also pancreatic secretion and gastro-intestinal tone and motility.

Over the last few months, the pace of advance in this field has increased considerably. The breakdown of the amino-acid sequence of the two peptides and their subsequent synthesis have been announced [38], followed by the preparation of a series of peptides structurally related to gastrin and possessing the spectrum of activity shown by the original peptides [4]. Still more recently a number of gastrins derived from various species including man have been isolated and characterised [39].

These exciting developments are destined to have the most far-reaching effect on the study of the intimate mechanisms of gastric secretion, and justify the extension of research to the study of these/

these processes in man.

CHAPTER II

PURPOSE OF THE THESIS

The purpose of this work is to study the action of gastrin on gastric secretion in man. The advent of this new and versatile stimulant afforded the opportunity of re-investigating several aspects of the secretory process.

The following were therefore studied:

(i) The spontaneous secretion over a prolonged period in normal subjects conditioned to the procedure.

(ii) The kinetics of secretion following various modes of administration of stimulants including gastrin.

(iii) The effectiveness of gastrin as compared with other stimulants and its use in secretory tests.

(iv) The dose-response relationships for gastrin.

(v) The cellular masses in the stomach as the determinants of the outputs of various secretory products.

(vi) The behaviour of electrolytes during steady and non-steady-states of secretion.

The fundamental studies dealing with dose-response relationships, the kinetics of secretion and the behaviour of electrolytes were conducted on two male medically-qualified subjects, JM (wt. 82 kgs) and GM (wt. 64 kgs), each of whom underwent nearly 50 tests over a period of two years.

The/

The comparative study of the action of gastrin and histamine on acid secretion was performed on a series of 16 normal and ulcer subjects, while the study of the simultaneous secretion of electrolytes, pepsin and total nitrogen was performed on a further series of 6 subjects.

Several subsidiary studies were also undertaken to determine the effectiveness of gastrin and the possible effect of antihistamine on the response to this stimulant.

All these series will be described more fully in the appropriate sections.

CHAPTER III

METHODS

In this chapter, the general procedures common to all secretory tests in man will be described; the more specific aspects of various tests will be dealt with under the appropriate headings.

All subjects under study were fasted overnight, and all anti-cholinergic agents withdrawn at least 24 hours beforehand. In the morning, a no. 7 radio-opaque Levin tube was passed via the nose into the stomach, and sited radioscopically so that its tip lay in the most dependent part of the stomach. The tube was then securely strapped to the subject's nose and forehead to ensure that there could be no displacement during the subsequent procedures. The tests were carried out in a quiet warm room, with the subject lying on his left side. In each test continuous individual attention was maintained throughout.

A. Collection of juice:

The quantitative study of gastric secretion requires the maximum of accuracy in the collection of juice. In these studies, subjects GM and JM collected the juice from each other and from all other subjects.

Continuous suction was applied at a subatmospheric pressure of

3-4 cm. Hg, supplemented as required by manual suction. The flow of juice was inspected throughout the test, with frequent interruption of suction and injection of air to ensure continuous patency of the tube. Early in these observations, the invaluable "pumping" effect of deep, forceful respiration, and abdominal straining was noted (Dr. B. Nordgren, personal communication), and thereafter this useful adjunct to the technique was utilised.

The secretion was collected for a period of 20 to 60 minutes prior to the test and labelled "basal". Following administration of the stimulant, aspiration was continued for a variable period depending on the nature of the test. Five-minute collections were taken throughout the test and their volume recorded. They were then pooled for titration into samples corresponding to 10-minute collections, except in tests involving single intravenous injections of gastrin, when 5-minute samples were estimated directly.

Frequent sampling of the juice is a pre-requisite of accurate collection and allows a close watch to be kept on the time of appearance of peak response and the emergence of a steady-state of secretion. The errors involved in longer periods of collection are more difficult to detect and may lead to an underestimate of the value of peak output which is the measurement best related theoretically and experimentally to the dose of stimulant.

Since the general tendency is for an earlier sample to be under-collected, the probable direction of error in collection is forward/

forward, shifting the position of peak output to the right and altering its magnitude.

Under-collection of samples is, of course, most likely to occur at low secretory rates, and unless guarded against introduces fallacies in the quantitative description of gastric electrolyte phenomena (see section on electrolytes).

B. Contamination of the juice:

Extragastric contamination is the other potential source of error in gastric studies in man.

(a) Biliary contamination:

In the studies reported here, biliary contamination varied with the individual. Its appearance tended, though not invariably, to coincide with the end of the first hour following stimulation. Although the volume appeared to increase concomitantly, the acid output was not significantly changed.

In subjects GM and JM, biliary contamination virtually ceased after the first few months of testing and occurred only in occasional samples following administration of cholinergic agents.

(b) Salivary contamination:

When the emphasis in a particular study is on the output of acid following stimulation, it is probably unnecessary to attempt to reduce salivary contamination.

In the study of gastric electrolytes, however, it is imperative to avoid such contamination. The technique used in these/

these studies which consisted in the application and frequent changing of dental pledgets in the sulci of the cheeks and under the tongue, appears to have ensured virtual elimination of this potential source of error. Incidentally, the technique proved to be a reliable method of quantitating the output of saliva and demonstrated clearly the absence of any stimulatory effect of gastrin on salivary secretion. In subject GM, during 17 tests in which gastrin was administered by single intravenous injection or by continuous infusion over a wide range of doses, the mean output of saliva was 32.8 ml/hr. $\pm$ 1.8 S.E. as compared with a mean output during basal tests of 28.4 ml/hr. $\pm$ 1.4 S.E. There was no significant difference between the two means.

It should be noted that contamination by bile, saliva or blood affects the composition of the juice differently. Biliary contamination increases the sodium content of a sample but decreases the concentration of other electrolytes. Salivary contamination, unless excessive, has little effect on the electrolyte content of a sample, but reduces the concentrations of all other constituents except potassium. Blood contamination has an insignificant effect on electrolyte composition but an important effect on the total nitrogen content.

C. Analytical Procedures:

The juice was always filtered with lint prior to all analytical procedures.

Hydrogen/

Hydrogen ion concentration was determined using 0.1 N NaOH with phenolphthalein as indicator. The volume of juice used for titration ranged from 1 to 5 ml. and varied with the size of the sample. Microtitration (Pye Rotary Burette) was necessary on occasion with the smallest samples (0.2 ml. of juice and 0.01 N NaOH).

In the context of acidity determination of gastric juice, the best estimate of total hydrogen ion concentration appears to be provided at a point of equivalence situated near the end-point of phenolphthalein (Moore and Scarlatta, 1965) [40].

Sodium and potassium concentrations were determined by flame-photometer (EEL model A).

Chloride concentration was determined electrometrically using a 0.2 ml. aliquot of juice. The sum of the concentrations of cations ($H^+ + Na^+ + K^+$) was closely similar in every case to the chloride concentration and rarely differed by more than 2 mEq/L.

Pepsin determination was carried out according to the method of Hunt [41], and expressed in pepsin units.

Total Nitrogen determination was carried out by a modified biuret technique (Gornall et al., 1949) [42]. The data obtained by the biuret technique follow the law of Lambert-Beer and can variously be expressed as optical density units, or following micro-Kjeldahl estimation of the standard as concentration of biuret-reacting nitrogen in mg/L.

D./

D. Definitions

- Secretory rate = volume output of juice per unit time.
- Concentration of acid and other electrolytes is expressed as mEq/L.
- Acid or electrolyte output = secretory rate in Litres x concentration in mEq/L.
- Pepsin or Nitrogen outputs = secretory rate in mls x concentration in units/Litre.

CHAPTER IV

SPONTANEOUS SECRETION

Spontaneous secretion was always studied prior to the administration of a secretory stimulant. All the subjects were tested in the morning, in quiet surroundings away from the ward and after an overnight fast.

Although the term spontaneous secretion is more accurately descriptive, it will be used interchangeably in this study with the more usual term basal secretion.

A. The spontaneous secretion in subjects GM and JM.

This study offered the unusual opportunity of studying the pattern of spontaneous secretion over a prolonged period in two normal subjects conditioned to the procedure.

In subject GM, the spontaneous secretion of acid in 37 samples collected over a period of 20 months oscillated over a narrow range: 0.88 mEq/hr. $\pm$ 0.34 S.D. The scatter of the data is illustrated graphically in Table I, which displays a near-normal distribution.

In subject JM, the behaviour of the spontaneous secretion during the first year of testing was similar to that found in subject GM. During this period, the mean acid output of nine collections was 1.35 mEq/hr. $\pm$ 0.25 S.D.. Over the subsequent eight months, nine further collections were tested and found to contain/

	Range	
	0.15 - 0.30	0.1
	0.31 - 0.45	0.3
	0.46 - 0.60	0.46 - 0.56 - 0.56
	0.61 - 0.75	0.61 - 0.61 - 0.63 - 0.68 - 0.69 - 0.69 - 0.71 - 0.74
	0.76 - 0.90	0.78 - 0.79 - 0.82 - 0.85 - 0.86 - 0.87
Mean 0.88	0.91 - 1.05	0.92 - 0.92 - 1.00 - 1.00 - 1.01 - 1.04
	1.06 - 1.20	1.08 - 1.08 - 1.09 - 1.13 - 1.15
	1.21 - 1.35	1.20 - 1.25 - 1.32
	1.36 - 1.50	1.35 - 1.44
	1.51 - 1.80	1.69
	1.81 - 1.95	1.85

TABLE I. DISTRIBUTION OF BASAL OUTPUT in mEq/hr in subject G. M. over a period of 20 months.

n = 38, mean 0.88 mEq/hr $\pm$ 0.34 S.D.

contain no acid. The average volume of these collections was 25.5 ml/hr., their pH ranged between 7 and 8, and on three occasions when it was tested for, the bicarbonate concentration was 1.2, 6.8, and 9.3 mEq/L. The pH of the fasting secretion that had accumulated overnight was also neutral or slightly alkaline.

Discussion and conclusions

Hollander[43] has demonstrated that the fasting juice collected from the denervated pouches of dogs with ablated antra is not only anacid but contains a measurable amount of bicarbonate. The situation in conditioned subjects appears to be analogous to that in dogs, which leads to the conclusion that under strictly basal conditions the hormonal or nervous influences acting on the acid secreting mucosa are either minimal or totally absent. It can indeed be said that the parietal cell population is not spontaneously active, but only reactive to stimulation.

B. Spontaneous secretion in the experimental series:

The data from all the series were pooled together for analysis. Forty-five subjects in all were tested. Of these, 10 were normal subjects and 35 had confirmed peptic ulceration (25 D.U., 10 G.U.). In most cases, two basal tests were performed and a total of 83 collections were obtained for analysis.

The following preliminary observations can be made:

(i) There was a tendency for the first test to show a higher acid output than the second. This is in conformity with the earlier/

Total No. of samples	Makhlouf 67		Kirsner et al 533		Grossman et al 1550		Kay 179		Edinburgh Group	
	n	mEq/hr	n	mEq/hr	n	mEq/hr	n	mEq/hr	n	mEq/hr
D.U. males	50	5.82	174	5.88	787	5.29	152	6.8	175	6.0
G.U. males	8	1.84	40	2.29	148	1.45	-	-	-	-
Normal males	9	2.28	319	2.57	615	2.44	27	2.2	14	2.5

TABLE I. Mean basal output mEq/hr. in various series.

earlier findings of Littman [44] and of Sun and Shay [45], whose observations, however, were mainly confined to patients with duodenal ulcers.

(ii) Tests performed in the wards by the nursing staff showed consistently higher basal outputs. These were usually the earliest tests on the subject and do not form part of this series. These unusually high basal collections may in part reflect incomplete emptying of the fasting contents of the stomach.

Analysis of the data:

(a) Because of the paucity of data on female subjects (10 in all), only the data on male subjects can be considered as reliable. Table II shows the close similarity between the data from this series and those obtained from the much larger series of Grossman et al. [46] (1550 males) and Levin et al. [47] (533 males). In all three series, the basal output of normal subjects is slightly higher than that of gastric ulcer patients and around half that of duodenal ulcer patients.

(b) In this as in other studies, the basal output data were widely scattered. The range for duodenal ulcer patients was between 0.2 and 24 mEq/hr. All outputs above 6 mEq/hr. were confined to this group, but no limit could be set below which it could be affirmed that duodenal ulceration did not exist. The range for gastric ulcer patients was 0.1 to 5.8 mEq/hr. and that for normal subjects/

subjects was 0.2 to 5.6 mEq/hr.

(c) Fig. 1 shows however that the scatter of data is not random but around a mean for both duodenal ulcer patients and normal subjects. The mean output read at 50%, is again twice as high for duodenal ulcer patients as it is for gastric ulcer patients and normal subjects. The sigmoid character of the curves, which are identical with the curves derived from the data of Grossman et al. [46], is apparent on logarithmic transformation.

The data in the same subjects for the post-stimulatory hour output following gastrin or histamine show a similar type of distribution (Fig. 2).

It is clear from Figs. 1 and 2 that despite a wide difference in the means, the basal and "maximal" outputs of ulcer patients and normal subjects show a considerable overlap.

(d) If the acid outputs following maximal subcutaneous stimulation by gastrin or histamine are grouped by decades and their means plotted against the means of the corresponding basal outputs, a linear relationship is apparent (Fig. 3). All the data but one above 40 mEq/hr. (over one-third) pertain to male patients with duodenal ulcers.

A similar relationship between stimulated secretion and basal secretion may be derived from the data of Grossman et al. [46] who used a submaximal dose of histalog (50 mg. s.c.), and the data of Levin et al. [47], who used a submaximal dose of histamine (20 ug/kg s.c.). In these two series, however, the data for ulcer patients

and/

and normal subjects of each sex are plotted separately (Fig. 4).

Discussion

The most appropriate definition of basal secretion is that given by Lim who describes it as "the juice secreted by the stomach in the absence of all intentional and avoidable stimulation." This ideal situation is rarely attainable in practice. Indeed, two antithetical elements characterise the pattern of spontaneous secretion in man: variability and reproducibility.

The basal output according to Kirsner et al. [48] who performed serial tests on normal subjects and ulcer patients varies from day to day and from week to week. The pattern, however, is reproducible in multiple tests on the same subject. In conditioned subjects this variability can be reduced to a minimum, and under truly basal conditions in the absence of all nervous or hormonal influences the gastric mucosa is totally inactive.

Spontaneous secretion is thus usually the product of variable extrinsic or intrinsic influences, which can be loosely described as stimulus, drive or "dose", acting on a quiescent and fixed background, the parietal cell mass.

Since "maximal" acid output is linearly related to the size of the parietal cell mass, the curves in Fig. 2 describe the distribution of the latter in the duodenal ulcer and control groups. The ratio of the mean acid output of normal subjects to duodenal ulcer patients is around 70%, which is closely similar to the ratio

as/

as given by Cox [49] of their respective parietal cellular masses.

Similar curves in Fig. 1 describe the distribution of the basal output data. Their interpretation is more complex, since basal output depends on the size of secretory cell mass and varies with the influences activating it. The close similarity in the shape of the curves, however, leads to the inference that these influences are evenly spread both within a group and in between the groups. In other words, there appears to be no supranormal drive solely active in duodenal ulcer patients as compared with normal subjects or gastric ulcer patients.

These conclusions were put to the test by plotting the means of the "maximal" acid outputs against the corresponding means of basal outputs. The rationale of grouping the data from all subjects irrespective of condition derives from the calculations of Shay [50] who showed that acid output per billion cells is similar for normal and ulcer subjects of either sex. The grouping of data according to basal output, on the other hand, is open to serious error, since it is the "maximal" acid output which is the index of the stable cellular mass of an individual, while the basal output is the product of a variable drive acting on a stable background. All the points in Fig. 3 lie on the same slope including the last two derived exclusively from data obtained in male duodenal ulcer patients. The same linear relationship is shown in Fig. 4 by plotting the data of Levin et al. and Grossman et al. for the ulcer and/

and control groups of each sex separately. Titration to the end-point of free acidity is probably responsible for the intercept in Fig. 4.

The existence of a fixed ratio in all subjects between basal and "maximal" acid outputs had already been pointed out by Hunt and Kay [51]. Their view was challenged by Sircus [52] whose data showed a higher ratio in duodenal ulcer patients. In a recent review [53] the data obtained by both groups have been harmonised to fit the prediction of Kay and Hunt. The challenge has been revived more recently by Baron [54] who noted a bimodal distribution in the basal output of duodenal ulcer patients and pointed to an important source of error in the calculation of a parietal component from contaminated basal secretion.

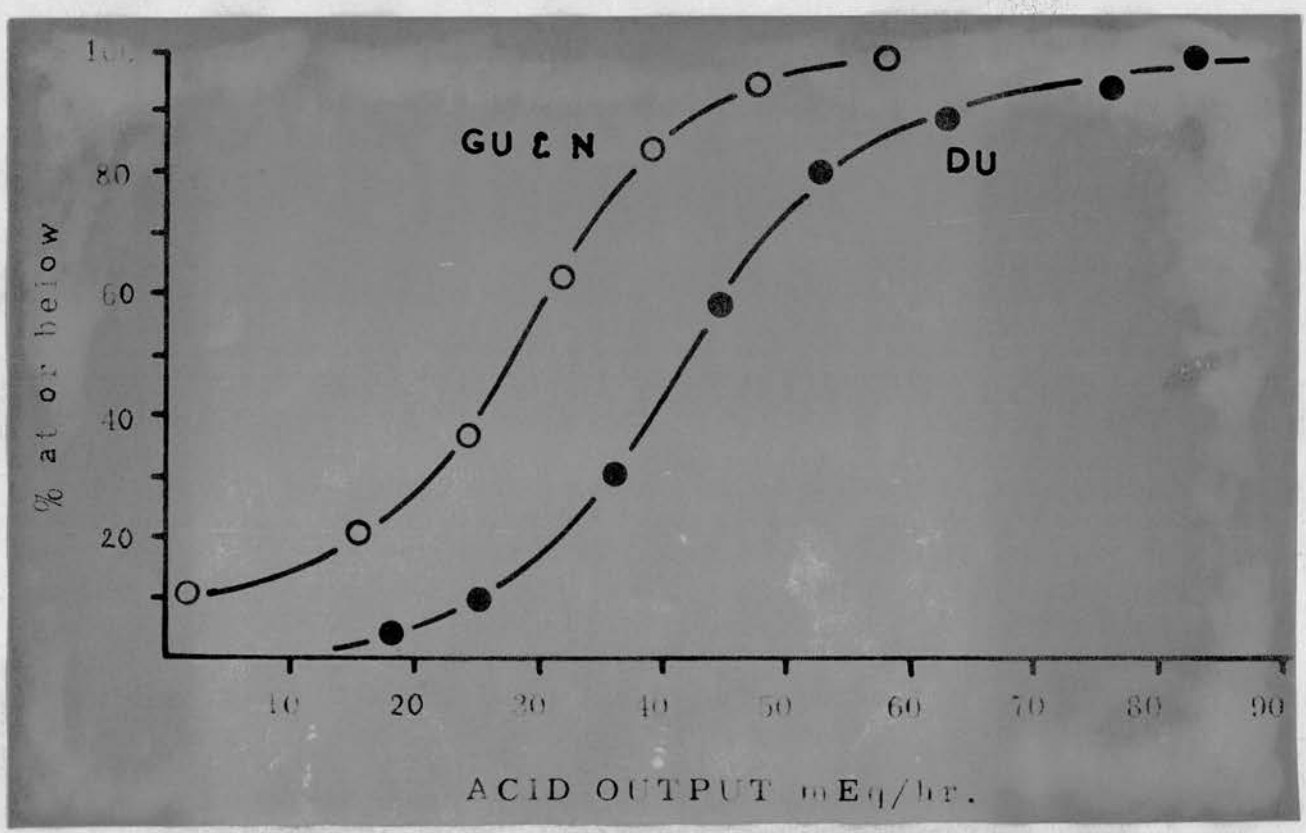
Despite the demonstrable existence of a fixed basal to "maximal" output ratio, it is nevertheless common experience that a higher than normal ratio is occasionally encountered in the highest secretors amongst the duodenal ulcer patients. Two questions may be asked: what is responsible for the higher ratio and why is it confined to some duodenal ulcer patients.

The answer to the first question lies in a consideration of the dose-response curve. The shape of the curve between 0 and 20% of maximal response is flat and near-linear. The ratio of basal to "maximal" output in all series is around 15 $\pm$ 5%. If the drive or "dose" is higher than a certain level, the response will lie on the second/

second and more vertical segment of the curve and the ratio is significantly altered.

It is not known whether the dose of endogenous gastrin released into the circulation differs in normal and ulcer subjects. Although a larger gastrin-bearing area has been reported in duodenal ulcer patients [55], this may only be a reflection of the larger size of their stomachs. This observation will assume greater importance, however, if it could be shown that in some duodenal ulcer patients the body cell mass and total body water into which a given dose of endogenous gastrin is released have not increased in proportion to the increase in the size of the gastric mucosa. Under these circumstances the effective dose of circulating gastrin would be larger than normal.

It is possible that the higher response is related to the presence of stenosis. A higher vagal tone leading to a greater release and more effective action of endogenous gastrin may be presumed to accompany this complication. The combined synergistic effect may well be responsible for a higher basal output with a mean value over 20% resulting in the significantly altered ratio occasionally seen in these patients.



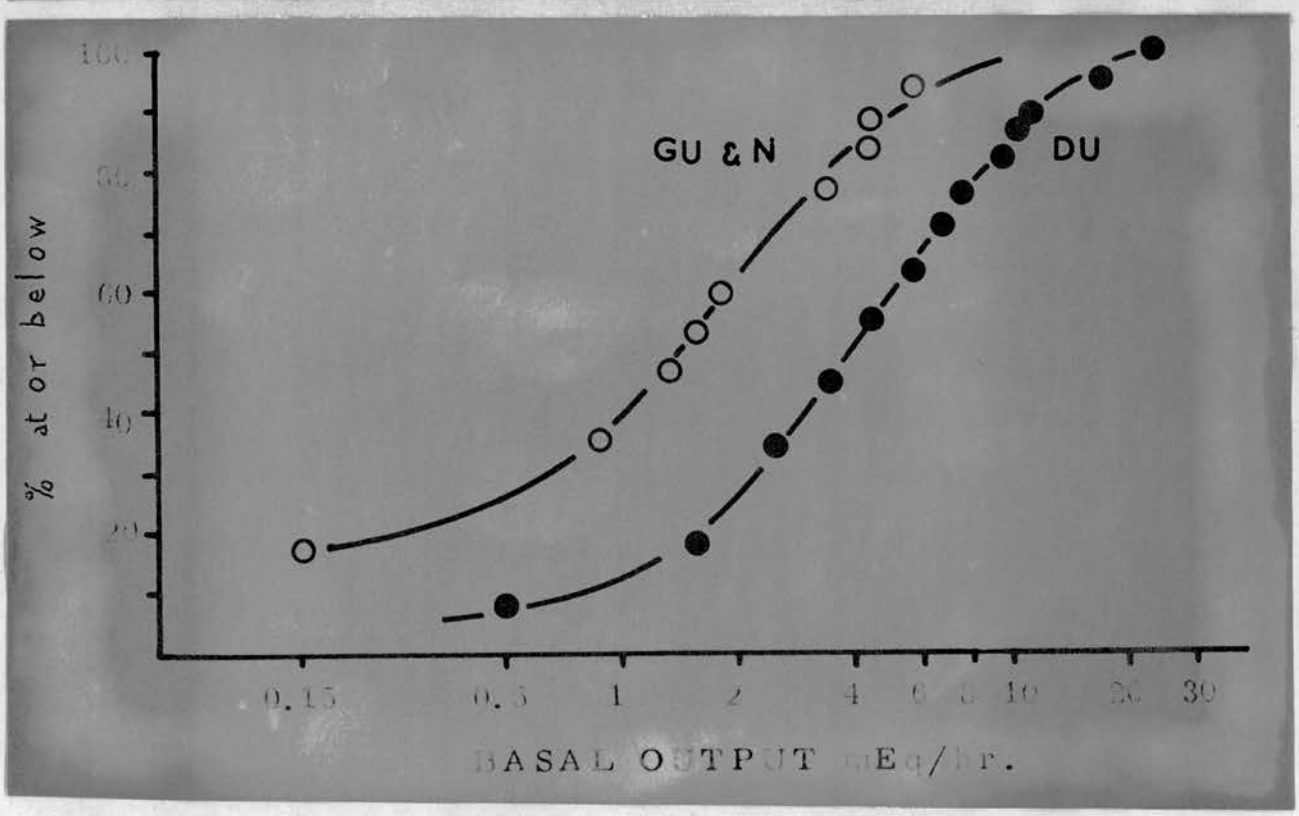


Figure 2: Cumulative distribution curves for post-stimulatory hour outputs in ulcer and normal subjects (above).

Figure 1: Cumulative distribution curves for basal output in ulcer and normal subjects (semi-logarithmic) (below).

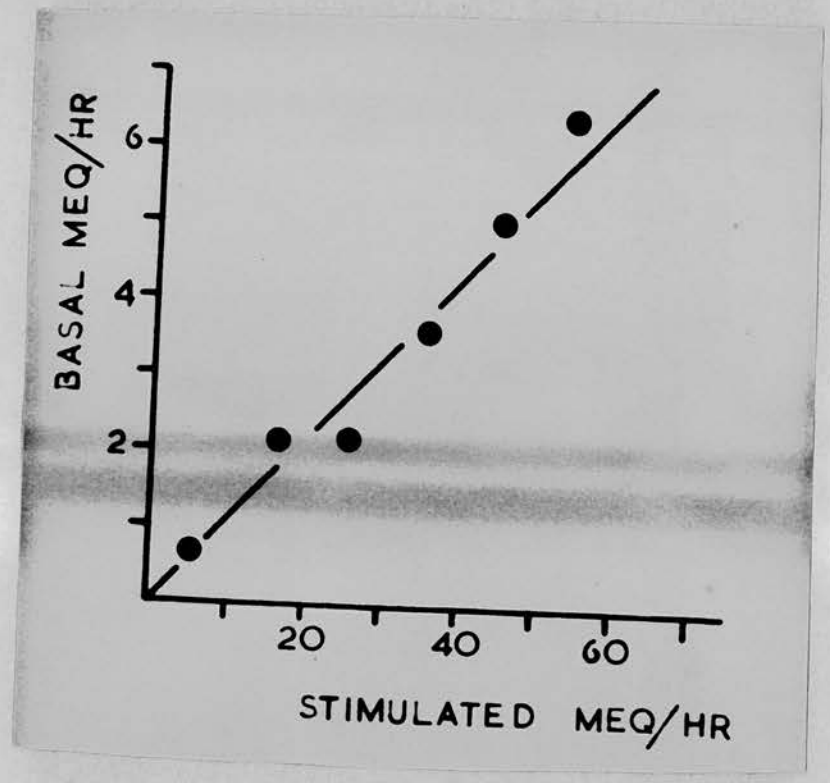


Figure 3: The relationship between the basal and post-stimulatory hour outputs in this study.

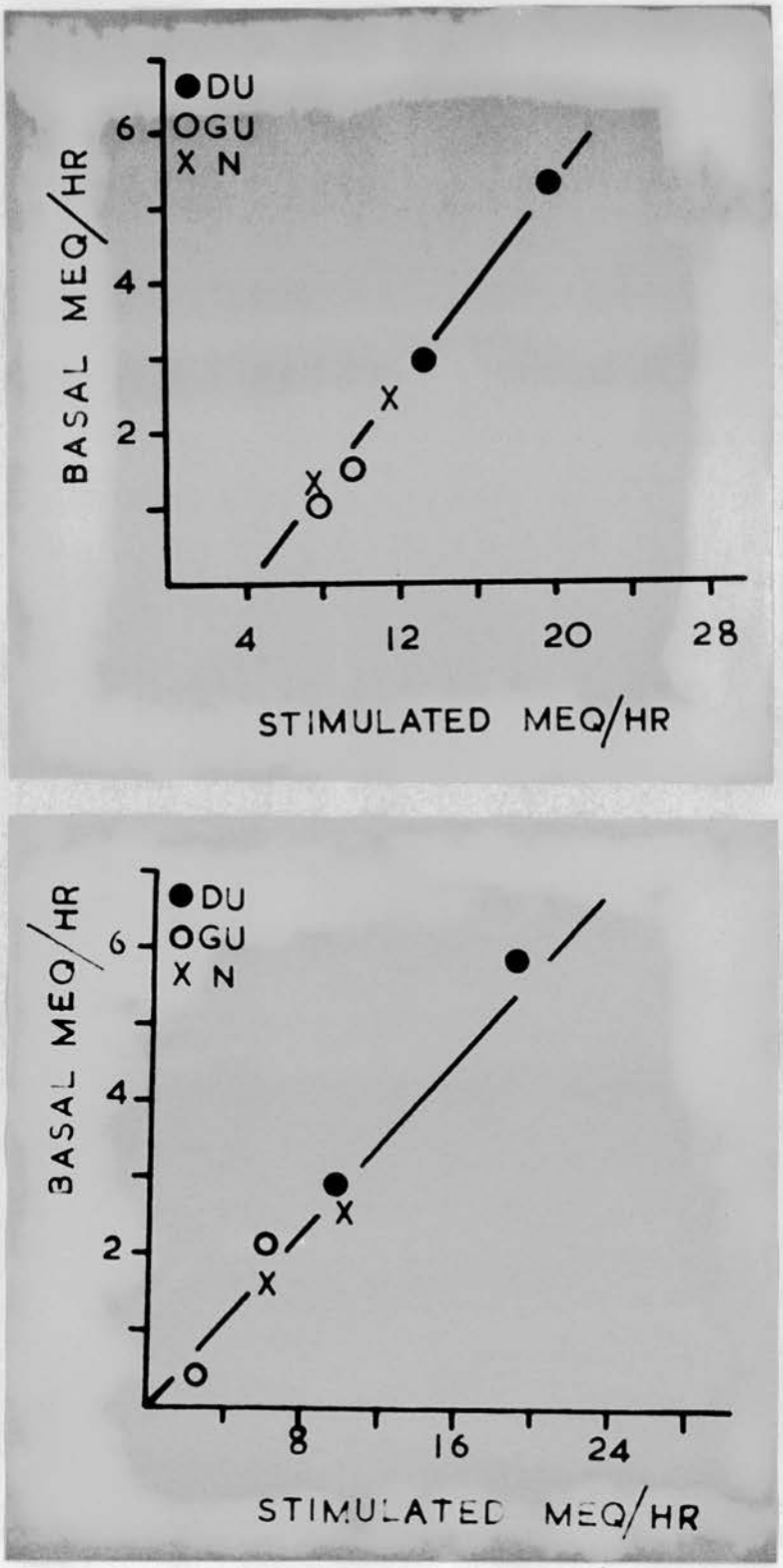


Figure 4: The relationship between basal and stimulated output in the series of Grossman et al (above) and Levine et al (below).

CHAPTER V

CONTINUOUS INFUSION OF GASTRIN

The purpose of this series of experiments was to investigate the pattern of secretory response to continuous infusion of gastrin and to establish dose-response relationships. The tests, which ranged over a period of one year, were conducted on subjects GM and JM.

A. Patterns of Response to Continuous Infusion of Gastrin and Histamine

Methods

The general procedures for intubation, collection of the juice and avoidance of salivary contamination were as outlined in the chapter on general procedures.

Gastrin II, dissolved in citrate buffer and its concentration adjusted to 10 ug/ml was placed in 1 ml ampoules. On the morning of each experiment the contents of one or more ampoules were washed out into a standard 50 ml syringe. Differential weighing of the ampoules permitted an accurate estimate of the dose extracted from them. The dose was varied by the number of ampoules used and by any subsequent dilution of the syringe contents. A constant speed of delivery of 11.2 ml/hr. was used in all experiments. Continuous infusion lasted for at least two, and usually for three hours, and the/

the doses used ranged from 0.2 to 90 ug/hr. Only one dose level was used in any single experiment.

The responses to continuous infusion of histamine acid phosphate were studied at the highest dose levels and mainly for purposes of comparison with gastrin. Two experiments were performed on each subject. In the first 40 ug/kg/hr. were infused for a period of two hours and in the second 20 ug/kg/hr. of histamine were combined with 0.06 mg/kg/hr. of mechothane and infused for a similar period. In both cases, 50 mg. of anthisan were administered prior to the start of the infusion.

Results

The acid secretory responses to a series of infusions with increasing doses of gastrin in subject JM are shown in Fig. 1. Except for the lowest dose levels, when a peak was slow to appear and difficult to detect, the pattern of secretion in both subjects was biphasic, with a peak output lasting 20 to 40 minutes depending on the level of the dose, followed by a near-steady-state response lasting for one to two hours.

In contrast, the response to continuous infusion of histamine or histamine plus mechothane shows a distinct difference in this respect (Fig. 2). The peak of acid output is only attained after some considerable time, usually around the end of the second hour. This slow rise was more evident in the response of subject JM, whose spontaneous secretion prior to the histamine infusion tests contained no acid.

Discussion/

Discussion

Frequent sampling of the juice is of great importance in the study of the kinetics or time-output relationships of secretion. The practice of taking five-minute collections permitted a close evaluation of the duration and magnitude of peak output and allowed a close watch to be kept on its time of appearance.

The difference between the patterns of histamine and gastrin is clearly of importance when maximal responses to the two stimulants are being compared. In this study, the infusion of histamine was not carried beyond 140 minutes. Hirschowitz, London and Pollard [56], who studied the secretory response to infusion of around 20 ug/kg/hr. of histamine over a period of three and a half hours, demonstrated a biphasic response to this stimulant with a peak of secretion around the end of the second hour followed by a steady-state response. A similar pattern to stimulation by histamine was also noted in dogs by Powell and Hirschowitz [57].

The biphasic response to infusion of gastrin is somewhat different from that of histamine and much more akin to that following infusion of urecholine [58]. Following infusion of gastrin, peak output appears early, around the middle of the first hour, and both its magnitude and its timing are dose-dependent. The most prominent rise and sharpest fall of output accompany the highest doses.

It was noted earlier that a near-steady-state of secretion appeared/

appeared during the second and third hours following stimulation by gastrin. A continuous decline in the response to infusion of gastrin has been noted in animals [58], and it is possible that in this study on man, minor errors in collection may have masked a similar but not very marked pattern of decline.

The pattern of secretory response is, at least in part, dependent on a similar pattern of distribution of the stimulant in the tissues, or more exactly in the target organ, the gastric mucosa. Teorell [59] was the first to consider on theoretical grounds, how the concentration of histamine in the blood and tissues might vary with the rate of infusion. Teorell assumed that the speed of cumulation in the tissues might depend on the difference between the rate of entry of the stimulant and its rate of disappearance by elimination or inactivation. When the rates were equal, cumulation ceased and the concentration remained steady and was proportional to the rate of infusion. Obrink [60] has since obtained evidence in support of this hypothesis.

When maximal secretory response to gastrin is being investigated it is more important to study peak output. The effective tissue concentration of gastrin responsible for this phase is also related to the rate of infusion (vide infra).

The study of the response to gastrin is further complicated by the possibility that it may not be directly responsible for stimulation, but acts by release of intracellular histamine [61]. The factors/

factors determining the effective concentration of the ultimate stimulant, - namely, the release of histamine from mucosal stores and its local synthesis, have both been shown in animals [61] to depend on the dose of gastrin. It thus appears sufficiently accurate to assume that the effective concentration of the ultimate stimulant is a simple and direct function of the infused dose of gastrin.

B. Dose-response Relationships following Infusion of Gastrin

Theoretical considerations:

(a) However accurate the data obtained, it is not possible to infer the mechanism of gastric secretion from the form of the dose-response curves. A number of models could be proposed each leading to curves consistent with the experimental observations. One possible model would be a population of secretory units each responding in a quantal or all-or-none manner but differing in threshold sensitivity to stimulation according to some defined frequency distribution. If as often happens pharmacologically the sensitivity to log-dose follows a normal distribution, a symmetrical sigmoid normal integral curve would be obtained. Three adjustable parameters define the curve: a vertical scale or height parameter, which is equivalent to the asymptotically maximal response at very high concentration; a location parameter identified with ED 50 or the dose eliciting half the maximal response, and a horizontal scale parameter/

parameter.

Alternatively it may be imagined that all cells or secretory units react uniformly, starting their secretion at the same dose but having a graded response to increasing doses. In this case, the shape of the dose-response curve for any individual cell is symmetrical and sigmoid, and maximal secretory response is represented by the sum of the activity of all cells.

(b) An alternative and more appropriate model may be proposed in which a number of secretory units or cellular receptors form active complexes with a well-defined number of molecules of the stimulant according to a reversible equation governed by the law of mass action. It is immaterial to the quantitative treatment that follows whether activation is considered to proceed during the entire period of receptor occupation or simply to occur at the instant of complex formation. It is assumed however that

(i) the number of secretory units or receptor sites is directly proportional to the number of parietal cells and that

(ii) response is proportional to the number of cells activated. For simplicity of treatment, "cell" is used in lieu of receptor or secretory unit.

Let,

C = total number of cells available for activation,

c = number of cells activated by cell-stimulant complex formation after a time t,

D/

D = concentration of the stimulant in the tissues during continuous infusion

C - c = number of cells in process of activation after time t.

The rate of activation or complex formation is proportional to the product of dose and number of cells in process of stimulation,

$$\frac{dc}{dt} = k_1 \cdot (C - c) \cdot D \qquad \text{Eq. 1}$$

The rate of deactivation or complex breakdown is proportional to the number of activated cells or formed complexes,

$$- \frac{dc}{dt} = k_2 \cdot c \qquad \text{Eq. 2}$$

At equilibrium, both reactions are proceeding at the same rate,

$$k_1 \cdot (C - c) \cdot D = k_2 \cdot c$$

Dividing both sides by k_1, and replacing k_2/k_1 by K,

$$(C - c) \cdot D = K \cdot c$$

$$C \cdot D = c \cdot (K + D)$$

$$c = \frac{C \cdot D}{K + D} \qquad \text{Eq. 3}$$

If c, which is identified with the observed response, is plotted against the logarithm of the dose, D, the formula gives the symmetrical sigmoid curve known as the logistic which is almost identical/

identical with the normal integral curve and is also fully specified by three parameters. The height parameter, represented by the maximal response following activation of all the cells C, is the same for the two curves. The location parameter or ED 50, is identified by the logarithm of K or the dose eliciting half the maximal response, and is again similar for the two curves. The scale parameters are analogous, but differ slightly in magnitude.

The analogy is apparent between Eq. 3 and other equations which can be fitted to logistic functions such as the Langmuir adsorption isotherms and enzyme-substrate reactions.

Further transformation of Eq. 3 uncovers a linear relationship between the reciprocals of response and dose,

$$\frac{1}{c} = \frac{1}{C} + \frac{K}{C} \cdot \frac{1}{D} \qquad \text{Eq. 4}$$

Since C or the total number of cells in any single stomach may be considered as constant, the relationship of 1/c and 1/D is linear. At infinitely high concentrations of stimulant, 1/c = 1/C.

(c) It is of interest to look back at the derivation proposed by Obrink [60] to account for the response of the stomach to stimulation. Obrink defined the secretory process as the "producing flow" which is converted into HCl by means of a stimulant; "a" represents the maximal response in ml/min. which will be secreted when all the "producing flow" is converted to HCl, while "v" is the rate at which the "producing flow" is converted into HCl/

TABLE I

Peak and plateau acid outputs in mEq/hr. and plateau volume output in ml/hr. following continuous infusion of gastrin II in subject JM (wt. 82 kgs.).

Date	Total Dose ug./hr.	Log-dose/hr.	Peak Output mEq./hr.	Plateau Output mEq./2nd hr.	Mean First Two Hours	Volume ml./hr. for Second Hour Plateau
28. 2.64	67.17	1.8261	51.50	43	42.19	306
19.11.63	12.73	1.1048	40.50	33.07	34.50	246
12.11.63	4.60	0.6628	26.36	21.88	21.70	200
16. 9.63	2.68	0.4292	17.98	15.24	16.11	128
30. 9.63	2.24	0.3500	15.84	13.94	13.71	132
7. 1.64	0.75	-0.1249	5.37	5.14	4.22	60
17.12.63	0.21	-0.6706	1.20	1.20	1.20	26.50

HCl following administration of a dose r. If r is increased by dr, this increment may react with the "producing flow" still left or (a - v), and cause an acceleration of secretion dv. Following the law of mass action, dv will be proportional to the product of (a - v) and dr,

$$dv = K (a - v) . dr$$

Integration of this equation gives

$$v = a (1 - e^{-Kr})$$

which is a well-known mathematical expression graphically represented by a curve passing through the origin and rising exponentially to an asymptotic value a. Logarithmic transformation of the dose r results in a symmetrical sigmoid curve. The similarity between Obrink's derivation and that proposed in section (b) above may be seen by substituting the symbols C or total number of cells, and c or number of activated cells, for a and v respectively.

Experimental data:

Table I gives the acid output values obtained from the peak response and from the second-hour steady-state (plateau) response in subject JM. All values in the table are expressed as per hour. The peak values have been calculated from the peak 20 to 40 minutes by multiplying this figure by the appropriate factor. Table I also gives the values of the volume output of the second-hour steady-state response.

Fig. 3/

TABLE II

ESTIMATES OF PARAMETERS OF DOSE-RESPONSE CURVES (LOGISTIC)

A

	Equation	H ± S.E.	b ± S.E.	a ± S.E.
Peak output	Y = 2.683X - 1.833	53.81 ± 0.309	2.683 ± 0.031	1.833 ± 0.0184
Plateau output	Y = 2.458X - 1.722	45.61 ± 0.532	2.458 ± 0.0656	1.722 ± 0.0309
Volumes (second hour plateau)	Y = 2.24X - 1.169	322 ± 14.99	2.24 ± 0.308	1.169 ± 0.131

B

Peak Output Values mEq./hr.		Plateau Output Values mEq./hr.	
Calculated	Observed	Calculated	Observed
51.42	51.50	42.90	43.00
40.68	40.50	33.27	33.07
26.17	26.36	21.73	21.88
18.08	17.98	15.46	15.24
15.62	15.84	13.54	13.94
5.53	5.37	5.30	5.14
1.39	1.20	1.51	1.20

Where H = maximal output, Y = logit, and X = $\log_{10}$-dose

Fig. 3 shows two plots of acid output per hour against log-dose. The upper curve represents the output of the peak achieved during the first hour and the lower one represents the steady-state values obtained during the second hour. Volumes obtained during the second hour plateau have been treated in a similar manner and are plotted in Fig. 4.

It is evident from each set of results that the response increases with the dose. The points have been fitted to a logistic function by minimising the sum of squares of residual deviations of the observed values. Table II gives the parameters of the curve with their standard errors. From the values of the parameters, a regression equation has been derived of the response in logits on log-dose.

Fig. 5 shows the linear relationship between the reciprocal of the dose and the reciprocal of either the peak or steady-state responses (Eq. 4).

Fig. 6 is a composite plot of the data obtained from both subjects. The dose is expressed per kg. body weight and the responses as percentages of the maximal calculated response of each subject.

Discussion

All the theoretical predictions which follow from Eqs. 3 and 4 appear to be well fulfilled by the experimental data.

Table II gives the standard error of the estimates, and the agreement/

agreement between the calculated and observed values gives an indication of the goodness of fit obtained. This fit indicates that the results are consistent with the secretory model on which the curve is based, but do not prove it, since, as has been noted above, the results could also be fitted to other functions based on other hypotheses of secretion. The goodness of fit, however, reflects the accuracy which may be achieved in the collection of juice from human subjects and the regularity of the response over a period of many months in a subject conditioned to the procedure.

It will be noted that the difference between peak and plateau response becomes greater with increasing doses of gastrin (Fig. 3).

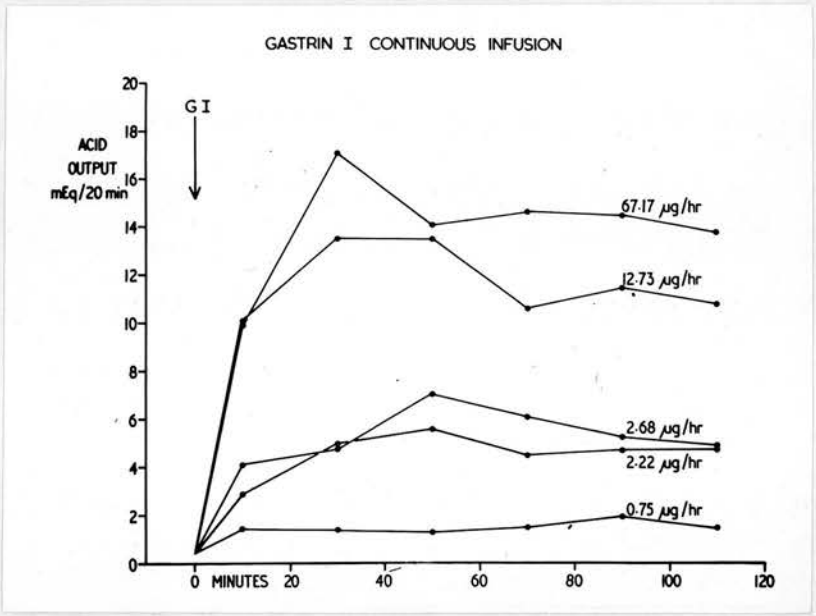
The volume output during the steady-state response when plotted against log-dose also shows a fair fit to the logistic function. Minimal extragastric contamination affects the volume considerably more than it does the acid output.

The location parameter, which determines the lateral shift of the whole curve, may depend in any single subject on the "reactivity" of the parietal cell to stimulation and/or on the rate of disappearance of the stimulant from the tissues. It appears from Fig. 6 that these factors are similar in subjects GM and JM since the ED 50, or dose eliciting half the maximal response is almost identical in both. This observation will gain further in importance when it becomes possible to estimate gastrin in body-fluids, especially in blood and urine, and relate the response of an individual/

individual stomach to the level of circulating gastrin.

The parameter which expresses the number of molecules of the stimulant which combine with a secretory unit is represented by the slope of the linear transformation of the logistic function (Table II, b). This parameter was calculated using the logarithm of the dose to base 10. If it is calculated to base e, then it will be seen to differ only slightly from unity. In terms of this model, this could be interpreted as indicating that the rate of the reaction between gastrin and secretory unit is mono-molecular.

The final suggestion of Adam et al. (1954) may yet prove to be true - namely, "that the height parameter, is the chief, and perhaps the only, parameter necessary to define gastric secretory output..." The accumulated evidence on this score in man [50, 62] and dog [63, 64] is impressive. The maximal secretory response, elicited on activation of the entire secretory cell mass, becomes the main and probably the sole discriminating measure between individuals.



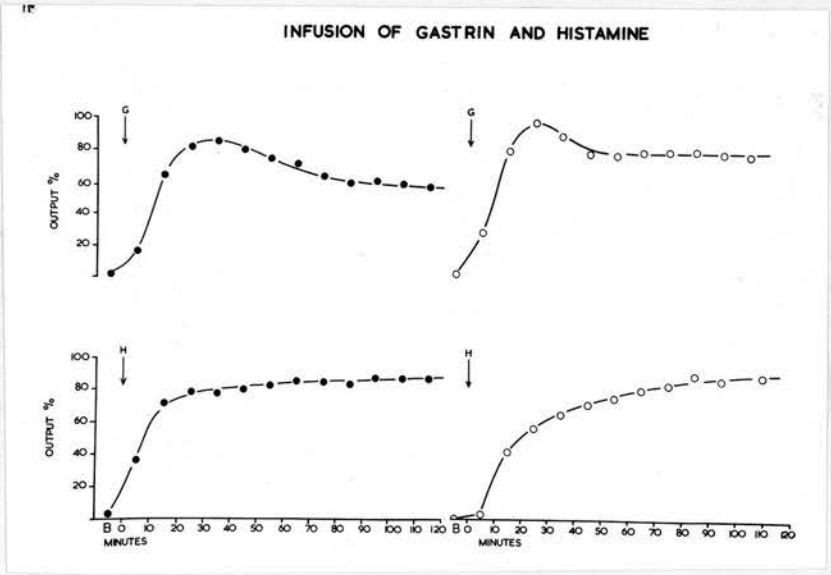
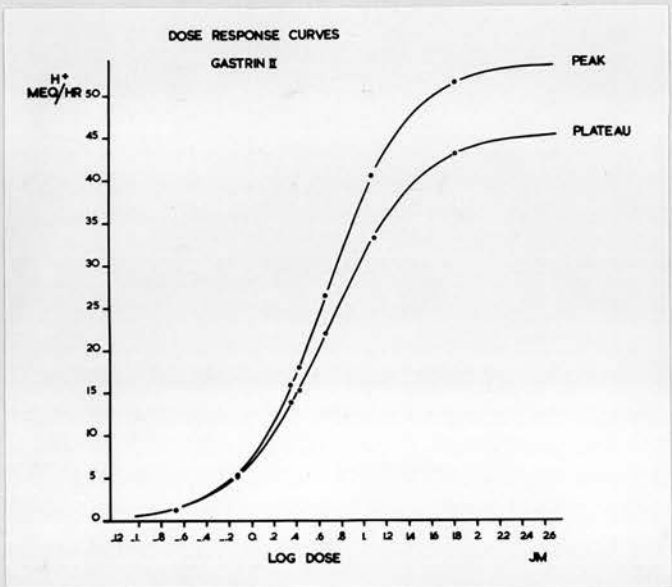


Figure 1: Plot of acid output in mEq/20 mins. following continuous infusion of graded doses of gastrin II (subject J. M.) (Above).

Figure 2: Upper graphs: the biphasic response to continuous infusion of a near-maximal dose of gastrin. Lower graphs: the response to continuous infusion of 40 ug/kg of histamine. Data from G. M. - closed circles; from J. M. - open circles.



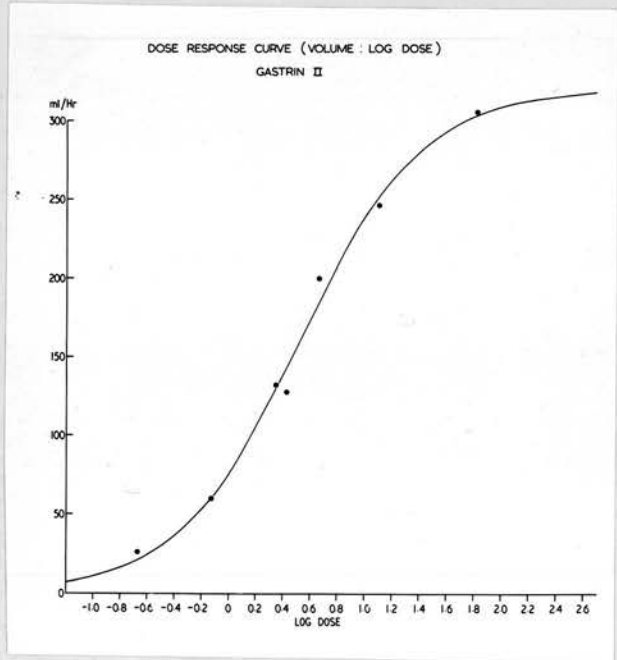
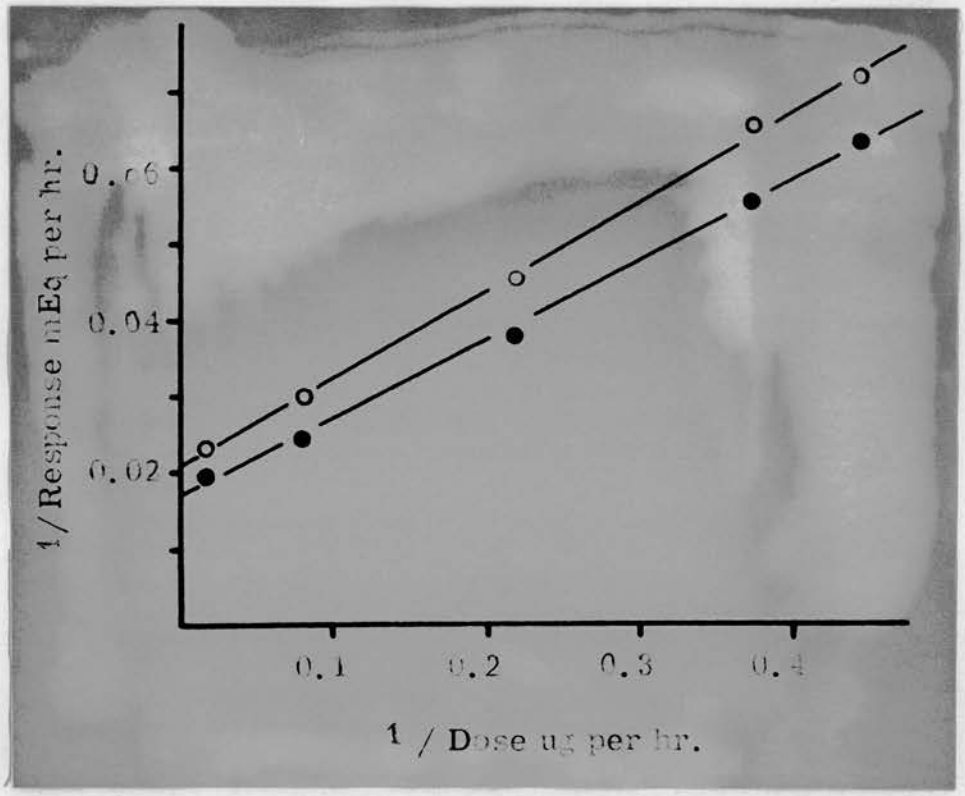


Figure 3: Dose-response curves of acid output in mEq/hr. against log-dose. Upper curve: peak output; lower curve: plateau output (above).

Figure 4: Dose-response curve of secretory rate during the second hour plateau against log-dose (below).



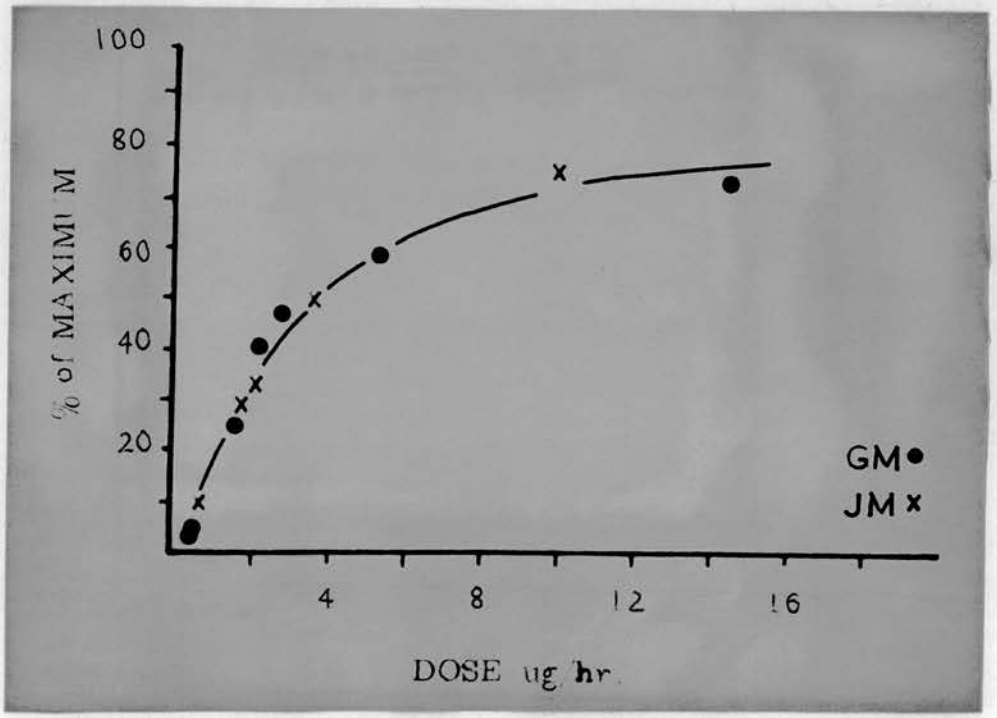


Figure 5: (above) Linear relationship between the reciprocals of dose and response. Open circles: plateau; closed circles: peak. The data are the same as in Figure 3 and Table I.

Figure 6: (below) Peak outputs of subjects JM and GM, expressed as percentages of their respective maximal outputs, against the dose per hour. The close correspondence of the values indicates that the location parameter is the same in both subjects.

CHAPTER VI

SINGLE INTRAVENOUS INJECTIONS OF GASTRIN

The preparation of pure gastrin made available for the first time a potent secretory stimulant that could be administered by prompt intravenous injection in man. Studies were therefore undertaken on subjects JM and GM to investigate the pattern of secretion and the dose-response relationships following this mode of stimulation.

Methods

The general procedures were as outlined in previous chapters.

The dose of gastrin was dissolved in 10 mls of distilled water. In the early trials, doses above 0.08 ug/kg were injected slowly over a period of three to five minutes. This was made necessary when a dose of around 0.25 ug/kg, injected rapidly, produced a sinking abdominal sensation which appeared within one minute and lasted for around five to ten minutes. Large doses, however, of up to 1 ug/kg, could be given slowly over a period of five minutes and repeated within ten minutes without eliciting any unpleasant sensations.

As further experience was gained in the use of intravenous gastrin, it was noted that more attention should be paid to the standardisation of the time over which the dose was delivered. Doses of up to 0.25 ug/kg were given thereafter, slowly and evenly, over a period of one minute, without discomfort.

Seven/

Seven experiments were performed on each subject. The range of doses employed in this study was 0.006 to 1.60 ug/kg. The order of the doses was randomised and the intervals between experiments were varied.

Six male ulcer patients (5 D.U., 1 G.U.) were also tested, for purposes of comparison, with doses ranging from 0.10 to 0.15 ug/kg.

A. Pattern of Secretion following Single Intravenous Injections of Gastrin

The characteristics of the response to single intravenous injections of gastrin are best illustrated graphically. Figure 1 shows the response of subject GM to a total intravenous dose of 4 ug (0.06 ug/kg). Latency was virtually absent and peak secretory rate was achieved within the second five-minute period, while peak acid output was achieved five minutes later and maintained for ten minutes. The secretory response lasted for nearly one hour. This pattern of secretion could be reproduced over nearly the entire range of doses, except that with the larger doses response lasted for up to 90 minutes.

Sampling of the juice at five-minute intervals allowed a good estimate of peak response to a particular dose to be made. The peak response was calculated from the peak ten-minute output, i.e. the sum of the two consecutive highest five-minute outputs, and the results expressed as the peak output per hour through multiplication by six.

The/

The pattern of response in the six ulcer patients was identical to that of subjects GM and JM (Fig. 2).

Discussion

The secretory rate, acid output and hydrogen ion concentration achieved their peaks in this order (Fig. 1). This is because the volume in the first two or three five-minute samples following stimulation is, to a large extent, of non-parietal origin. This question will be discussed more fully in the chapter dealing with electrolytes.

Administration of gastrin by single intravenous injections, results in a response which is the opposite extreme of a steady-state response. It is imperative, in view of the rapidly changing secretion, that the collection of juice be as accurate and as frequent as possible, if a meaningful statement of kinetic and dose-response relationships is to be obtained.

A short latency appears to be a property shared by gastro-intestinal hormones [65]. Recent experiments by Amure and Ginsburgh [66] on rats demonstrated a short latency following intravenous administration of crude gastrin extract and a virtually undetectable latency following gastric intra-arterial administration. The studies in man confirm these findings. Following intravenous injection of 3 ug of gastrin II over a period of 30 seconds in subject JM, secretory flow was evident within two minutes. The volumes/

volumes of the first three five-minute specimens were equal and were followed by a slow decline (Fig. 3). If account is taken of the circulation time from forearm to stomach, secretion would appear to have started in less than a minute and a half.

The plot in Fig. 2 is designed to compare the patterns of secretion in normal (GM and JM) and ulcer subjects. In both groups, mean acid output is expressed as a percentage of the respective peak output. The secretory curves which result from this treatment may be almost exactly superimposed. The close similarity in the shape of the curves from the two groups reflects, in all probability, the similarity in the patterns of distribution and metabolism of the stimulant in various subjects.

All the subjects in this study were also tested with maximal subcutaneous doses of histamine and/or gastrin. The response, during the first half-hour, to the intravenous dose employed (0.10 to 0.15 ug/kg) was of the same order as the maximal subcutaneous gastrin response.

B. Dose-response Relationships

Preliminary considerations:

The problem of establishing an accurate dose-response relationship following single intravenous injections of gastrin appears, at first sight, more complex than for continuous infusion. A measure of acid output must be devised that could be related to the/

the dose of gastrin, or more accurately, to the concentration of the stimulant in the target organ.

Teorell [59], who formulated the kinetics of distribution of a drug in the tissues following various modes of administration, demonstrated that a prompt single intravenous injection could be considered as a limiting case of a subcutaneous injection in which the resorption rate from a tissue depot is rapid. He derived two conclusions. First, that the time of appearance of peak concentration of a stimulant in the tissues for a particular dose, and by inference, of peak response, is independent of the injected dose; and second, that the magnitude of this tissue concentration and response is proportional to the dose.

The general validity of the first conclusion with respect to intravenous gastrin may be verified by inspection of Fig. 4. If output is calculated in successive ten-minute periods, the highest level, in all subjects and over the entire range of doses, invariably appears in the second ten-minute period. The actual peak output, i.e. the sum of the two highest consecutive five-minute outputs, usually coincides in time and magnitude with this level, but may on occasion occur five minutes later.

The validity of the second conclusion may be inferred indirectly from plotting the peak output against the logarithm of the dose. For if this conclusion is correct, it should provide a quantitative link between peak output and the injected dose of gastrin, and permit/

Date	Dose ug.	Log-dose.	Peak output mEq/hr.	First 20' output mEq.
		SUBJECT GM., wt. 64 kg.		
3. 4.64	100.0	2.000	36.00	7.9
3. 1.64	27.5	1.439	34.23	7.7
9.12.63	10.4	1.017	29.15	7.2
18.12.64	4.0	0.602	20.69	4.6
9.12.65	3.0	0.477	17.24	3.9
23. 2.65	2.15	0.332	14.60	3.8
9. 3.65	1.1	0.477	8.43	2.2
		SUBJECT JM., wt. 82 kg.		
13. 3.64	50.00	1.699	52.14	12.8
11. 3.64	30.00	1.477	46.20	12.4
10. 1.64	18.80	1.274	45.12	12.9
6. 1.64	4.66	0.668	31.38	8.2
2.12.64	3.11	0.493	27.24	8.1
17. 4.64	3.00	0.477	24.18	6.7
21.12.63	0.56	-0.251	7.38	1.6

TABLE I. Total intravenous doses of gastrin and the corresponding peak outputs in mEq/hr. in subjects GM and JM.

mit a fit of the secretory data to the same logistic function as derived in the preceding chapter.

Experimental data:

Table I gives the doses and corresponding peak outputs from a series of experiments in subjects GM and JM.

Fig. 5 shows a fit to a logistic function of the data obtained from subject GM. A similar fit is obtained from the data of subject JM.

If the doses are expressed per kilogram body weight and the corresponding peak outputs are expressed as a percentage of the calculated maximal response of each subject, the two curves may be exactly superimposed (Fig. 6).

Figure 7 shows the linear relationship expected between the reciprocals of dose and response.

Discussion

The good fit of the data to a logistic function confirms the prediction of Teorell regarding the relationship between peak output and the intravenous dose of gastrin.

Several important conclusions may be drawn from the dose-response curves:

(1) It was noted in the previous chapter that when a sufficiently high dose of stimulant is given, all secretory units available for activation are considered secreting. This theoretical level of secretion is represented by the asymptote of the sigmoid/

sigmoid dose-response curve and is an index of the secretory unit mass and, by extension, of the parietal cell mass.

The maximal responses calculated from the asymptotes of the dose-response curves following single intravenous injections are 36 and 54 mEq/hr. for subjects GM and JM respectively, and are subsequently referred to as the calculated maximal responses. These are identical with the calculated maximal responses for the dose-response curves described in the previous chapter and derived from continuous infusion of gastrin. This is to be expected if, as the model predicts, maximal response is essentially determined by activation of all available secretory units independently of how this is achieved. These maximal responses have, moreover, been experimentally reproduced in both subjects over a short period of 10 to 20 minutes by slow intravenous administration of a single massive dose of gastrin (50 to 100 ug). They represent in all probability the maximal secretory capacity of the intact human stomach to stimulation. It will be shown in a subsequent chapter that no other stimulant or combination of stimulants could be found that would induce a higher response.

(ii) The close correspondence of the curves derived from the data of the two subjects (Fig. 6) indicates that their location parameters are identical. This confirms the conclusions of the previous chapter that the distribution and metabolism of the stimulant and the reactivity of the parietal cells to stimulation are similar in different individuals.

It/

It would appear from inspection of the curve in Fig. 6 that the magnitude of the dose that would elicit half the maximal response is of the order of 0.045 ug/kg ± 0.005. A dose of 1 ug/kg is near-maximal and elicits a response around 95% of the calculated maximum.

(iii) Here, as in the previous chapter, the finding, in both subjects, that the value of the parameter which expresses the number of molecules combining with the postulated secretory unit is close to unity, indicates that the rate of the reaction between gastrin and secretory unit is mono-molecular.

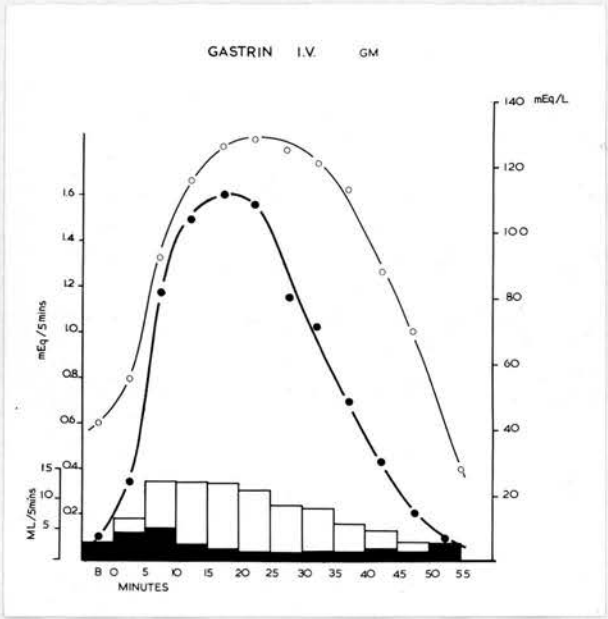
(iv) It is of interest to note how very potent is gastrin II when given in a single intravenous injection. Thus the administration of a total of 1 ug in subject GM results in a response which is around 20% of the calculated maximum (Fig. 5). If the level of circulating gastrin is responsible for the spontaneous secretion of the intact human stomach (2.5% of the maximal in both subjects), it must be of a very low order and corresponds, in effect, to an intravenous dose of around 2.5uug/kg.

(v) Recent experiments by Johnston et al.[67] appear to show the presence following a meal of a stimulant in the blood of human subjects. Re-transfusion in the same subject of 500 mls of blood obtained post-prandially doubles the basal acid output for a short period. If gastrin were responsible for the observed increase in secretion, the dose required to double the basal output on the basis of the curves derived in this study, would be of the order/

order of 4 to 5mg/kg (around 0.25 to 0.30 ug total). This dose should be present in the extracted volume of blood or 500 mls. Assuming gastrin to be freely diffusible in total body water of around 50 litres, and the 500 mls of blood a representative sample, the endogenous release of gastrin would be of the order of 25 to 30 ug delivered into the circulation post-prandially. Assuming further that antral release of gastrin is fairly rapid, 25 to 30 ug delivered into the circulation should result in a near-maximal response of over 90%.

It thus appears probable that the post-prandial response in man is near-maximal, at least for a time. In addition, the vagal effect of distension by a meal would probably lead to a synergistic effect. The simultaneous administration of 5 mg of mechothane subcutaneously in subject GM was sufficient to raise the response from a total dose of 6 ug of gastrin to nearly 80%.

(vi) It may be said, finally, that because of its simplicity and accuracy, the method illustrated in this chapter is potentially of great value in the investigation of gastric secretory function.



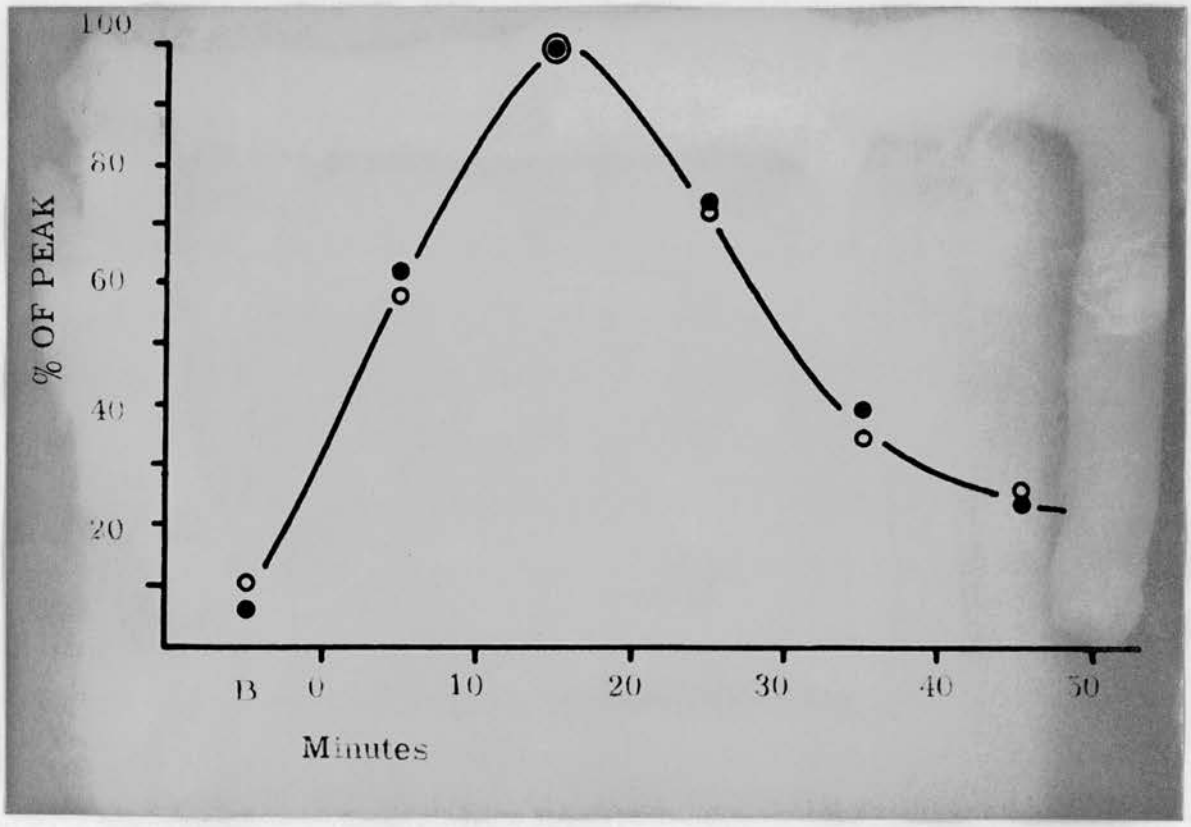
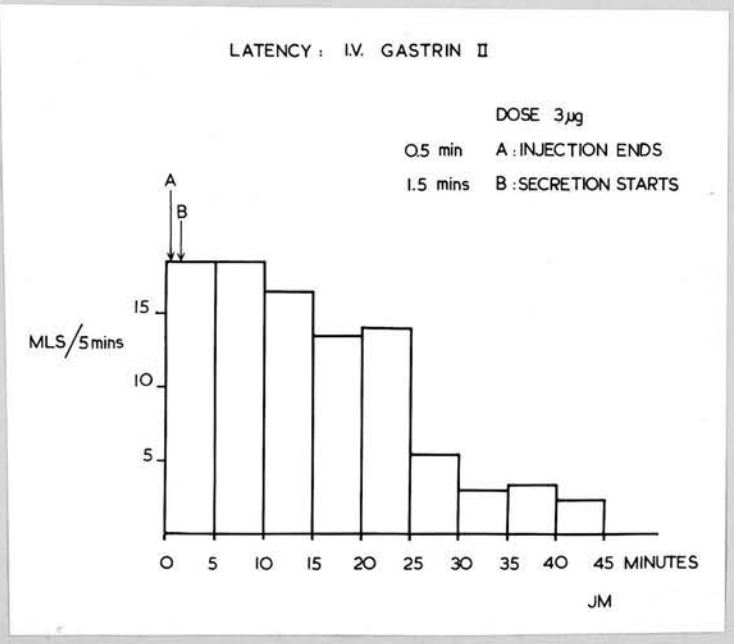


Figure 1: (above) Pattern of response to intravenous injection of gastrin. The blacked-out part represents the volume of the non-parietal component.

Figure 2: (below) Mean acid outputs of subjects GM and JM (closed circles) and six ulcer patients (open circles) expressed as percentages of their respective peak outputs.



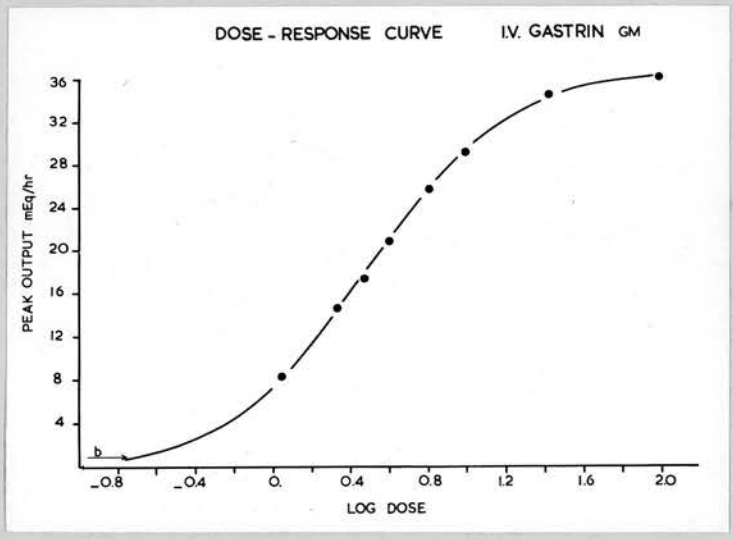


Figure 3: (above) Short latency to intravenous gastrin.

Figure 4: (below) Plot of peak output in mEq/hr against the logarithm of the total intravenous dose (subject GM).

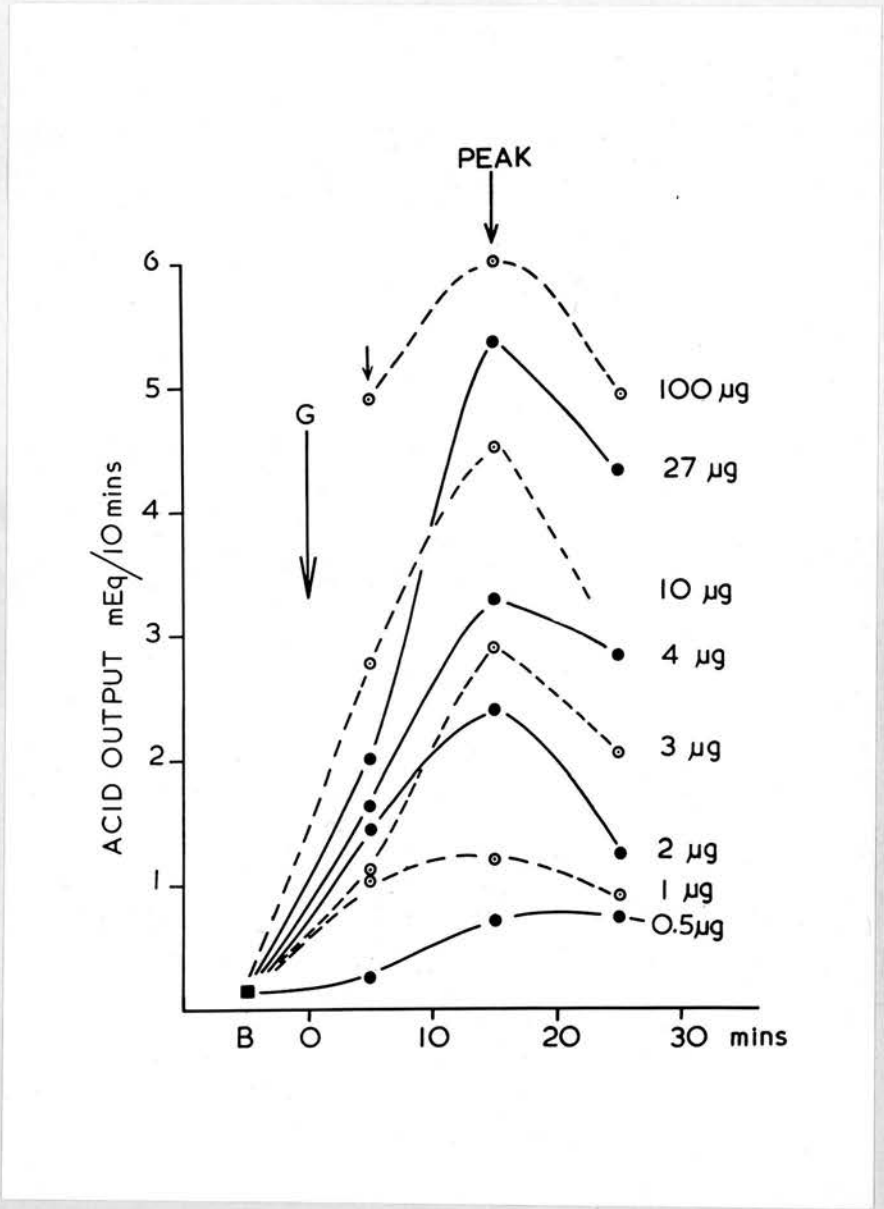
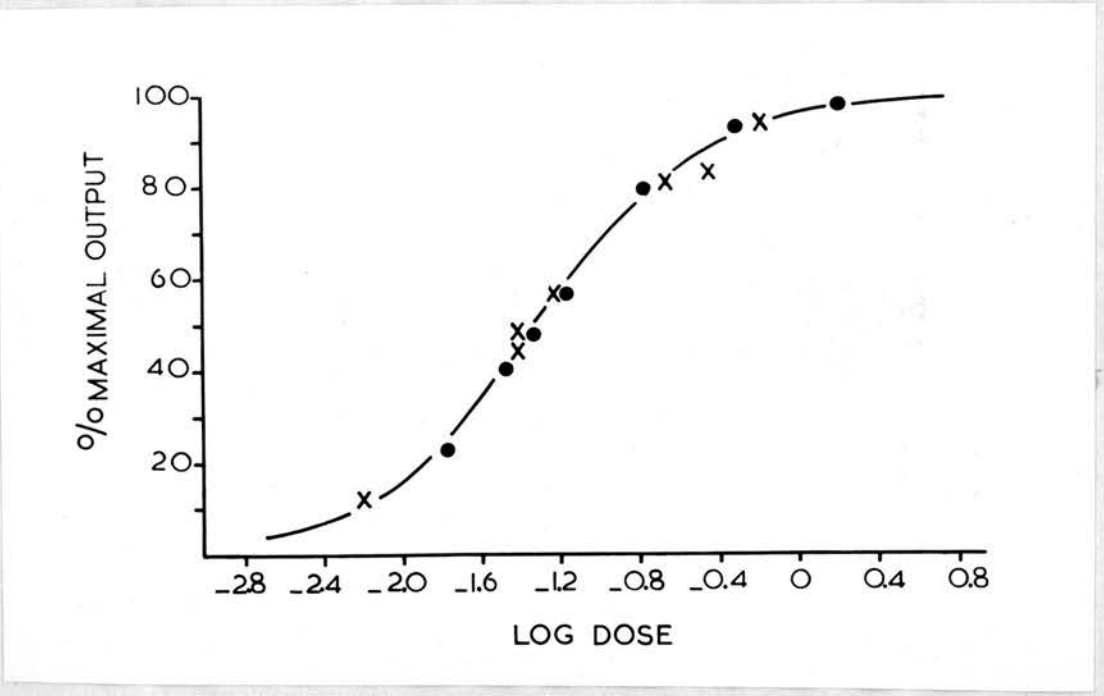


Figure 5: The time of appearance of peak output in the second 10-minute period is independent of the intravenous dose of gastrin.



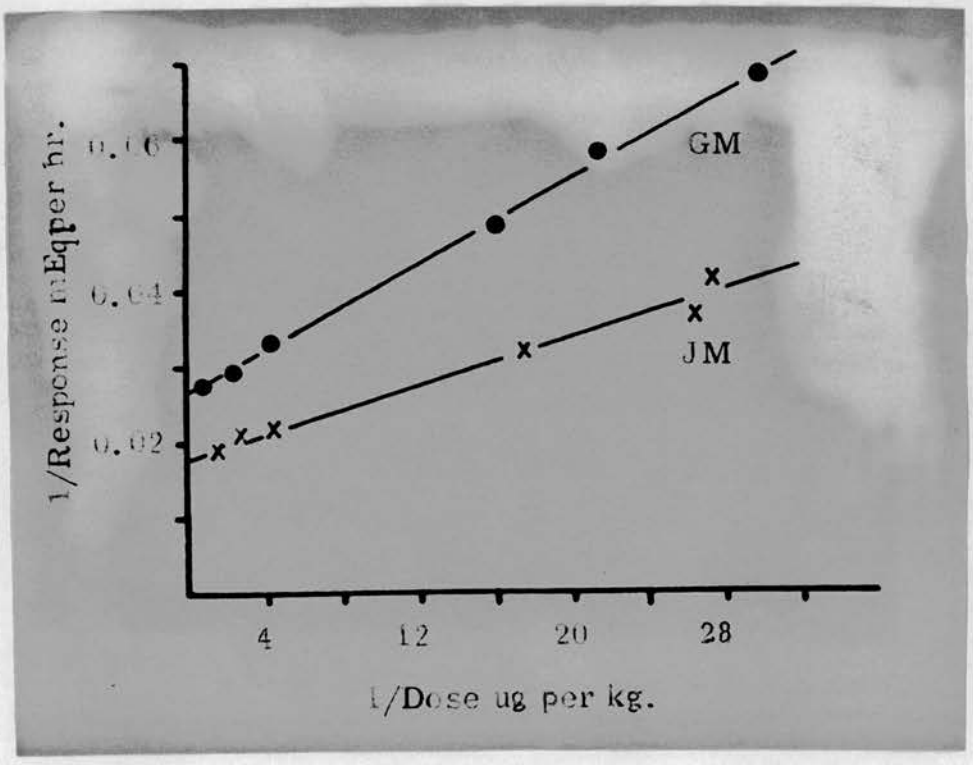


Figure 6: (above) Plot of peak outputs expressed as percentages of the respective calculated maximal response against the logarithm of the dose per kg. The location parameter (ED 50) is the same in the two subjects.

Figure 7: (below) The linear relationship between the reciprocals of dose and response. The intercepts on the vertical axis represent the reciprocals of the calculated maximal responses.

CHAPTER VII

THE EFFECT OF SUBCUTANEOUS ADMINISTRATION OF GASTRIN II

The purpose of this series of experiments was (i) to study the kinetics of secretion following subcutaneous administration of gastrin, (ii) to evaluate the potency of gastrin in relation to histamine and establish the maximal subcutaneous dose, and (iii) to devise a stable and reliable measure of gastric secretory activity.

Methods

The general procedures were as outlined previously. Since the emphasis in this study was on acid output following maximal stimulation, no effort was made to minimise salivary contamination.

Secretory tests were conducted on three sets of normal and ulcer subjects:

(i) One normal and five duodenal ulcer subjects were each given on different days, subcutaneous injections of 40 ug/kg of histamine acid phosphate and 0.5 ug/kg of gastrin II. The injection of histamine was preceded by the intramuscular injection of 100 mg. of mepyramine maleate.

(ii) Two normal subjects, GM and JM, and one duodenal ulcer patient were given progressively increasing doses of gastrin II to establish the subcutaneous dose that would elicit a maximal secretory response.

(iii)/

(iii) Sixteen subjects (6 D.U., 5 G.U., 1 P.A., and 5 normal) were each tested on different days with 40 ug/kg of histamine and 2 ug/kg of gastrin II. All peptic ulcer patients had their diagnosis confirmed at operation. Subject 15 (Table A), a low secretor, was a healthy male with no family history of disturbance of gastric function or thyroid disease.

In all instances, gastrin was injected by a standard technique into the lateral aspect of the right upper arm. An interval of at least two days was allowed between tests on any single subject, and the order in which the tests were carried out was randomised.

Spontaneous secretion was collected for an hour prior to the test. Aspiration was continued for 70 minutes after histamine and for 120 minutes after gastrin. During the test, 5-minute collections were taken and pooled into samples corresponding to 10 minutes for purposes of titration.

The degree of biliary contamination varied with the individual. Its appearance tended, though not invariably, to coincide with the end of the first hour. Although the volume appeared to increase concomitantly the acid output was not significantly changed.

The first seven 10-minute samples in the case of histamine and all twelve samples in the case of gastrin were titrated with 0.1N NaOH, using phenolphthalein as indicator.

Two estimates of acid output were made in these studies:

(i) the total output in the post-stimulatory hour,

(ii) the peak hour output which was calculated from the second 20-minute peak output through multiplication by three.

Side-effects/

Side-effects

There was complete absence of side-effects on subcutaneous administration with the whole range of doses of gastrin in every subject tested. A few subjects had noticeable borborygmi and increased passage of flatus during the latter stages of the test and for one or two hours after.

A. Pattern of Secretion following Subcutaneous Administration of 0.5 ug/kg of Gastrin

The characteristics of the response to this dose of gastrin are illustrated in Figs. 1 and 2, and Table I. The onset of secretion was fairly rapid but the rate of increase was somewhat more gradual than with histamine (Figs. 2 and 3). The peaks of volume and acid output were achieved simultaneously during the fourth to fifth 10-minute period, that is some ten minutes later than with histamine, and were followed by a slow decline. Collection of the juice was carried out for two hours only. But both the shape of the secretory curve and the level of secretion at the end of the second hour - 60% of peak output - indicate that stimulated secretion would have been maintained for much longer.

Despite the slower rise of secretion as compared with histamine, the peak and post-stimulatory hour outputs achieved under both modes of stimulation were substantially similar (Table I).

Table II gives the mean values for the six subjects tested and provides a summary of the findings.

B./

Table I. Acid output in mEq. per 10 min. in 6 subjects following subcutaneous administration of gastrin II 0.5 ug. per kg. and histamine acid phosphate 40 ug. per kg.

Values underlined represent peak values of acid output per 10 min.

Subject No.	1	2	3	4	5	6
Histamine 40 ug/kg s.c. Volume (ml./10 mins.)						
1	57	38	39	38	18	13
2	74	53	45	45.5	28	29
3	78	52	45.5	59.5	31	32
4	78	60	42	56.5	29	32
5	73	48	43	60.5	29.5	34
6	73	47	39.5	52.5	27	32.5
7	74	33	32.5	45.5	25	34
Post-histamine hour	433	298	254	312	162	172
"Peak hour"	450	330	263	353	176	197
Histamine 40 ug/kg s.c. Acid output (m.Eq/10 mins.)						
1	5.13	4.18	3.27	3.00	1.55	0.40
2	8.36	6.51	5.19	5.30	3.48	1.00
3	9.51	6.90	5.58	7.14	4.26	2.34
4	9.75	6.78	5.57	7.06	4.07	2.51
5	8.46	6.16	5.58	7.00	4.16	2.95
6	8.79	5.85	4.78	6.35	3.45	2.53
7	8.47	3.81	3.55	5.59	2.41	2.41
Post-histamine hour	50.00	36.38	29.97	35.85	20.97	11.73
"Peak hour"	57.78	41.04	33.46	42.40	24.98	15.98
Gastrin 0.5 ug/kg s.c. Acid output (mEq/10 mins)						
1	1.92	2.97	1.21	1.03	0.93	0.02
2	7.75	5.56	2.07	4.46	1.87	0.21
3	9.47	7.01	5.00	6.45	3.40	2.03
4	9.03	7.23	6.74	7.73	4.06	3.74
5	8.82	7.23	7.10	7.73	4.69	3.33
6	8.39	7.28	7.08	6.18	4.00	3.56
7	8.97	6.60	6.62	6.18	2.97	3.48
8	8.51	5.74	6.26	6.12	1.87	3.78
9	6.21	5.74	6.92	6.02	2.09	2.92
10	6.32	5.71	6.52	5.36	1.61	2.98
11	6.66	5.24	6.03	4.17	1.61	2.89
12	6.02	5.01	6.00	4.04	1.54	2.60
Post-gastrin hour	45.38	37.28	29.20	33.58	18.95	12.89
"Peak hour"	55.50	43.13	42.54	46.38	25.50	21.47

TABLE II. Mean acid output, secretory rate and concentration per 10 minutes following subcutaneous administration of 0.5 ug/kg of gastrin (left) and 40 ug/kg histamine (right).

10-minute periods.	Volume mls.	H^+ mEq/L	Acid output mEq.	Volume mls.	H^+ mEq/L	Acid output mEq.
1	19.5	68.5	1.34	34.0	86.0	2.92
2	38.0	95.5	3.65	45.5	105.0	4.80
3	50.5	110.0	5.55	49.5	120.0	5.95
4	56.0	114.0	6.42	49.5	120.0	5.95
5	59.0	109.0	6.48	48.0	119.0	5.71
6	52.0	117.0	6.08	45.0	117.0	5.29
7	52.0	111.0	5.80	40.5	107.5	4.37
8	46.0	116.0	5.38			
9	42.0	118.0	4.98			
10	41.0	116.0	4.75			
11	39.0	113.0	4.43			
12	36.5	115.0	4.20			

B. Maximal Subcutaneous Dose of Gastrin

The peak response to 0.5 ug/kg of gastrin, though substantially similar in magnitude to the peak response after stimulation with histamine, does not represent the maximum of which the stomach is capable in response to gastrin. It is clear from Fig. 4 and Table III that the post-gastrin hour outputs were higher with 2 ug/kg than with 0.5 ug/kg or 1 ug/kg. All six subjects tested with 0.5 ug/kg of gastrin were subsequently tested with 2 ug/kg and invariably showed a rise in acid output of around 10 to 15%.

In an attempt to establish the dose of subcutaneous gastrin that would elicit a maximal response, increasing doses of up to 4 ug/kg were given to three male subjects. Responses to 3 and 4 ug/kg were essentially similar to the response obtained from 2 ug/kg (Fig. 4). It thus appears that 2 ug/kg of gastrin represents the subcutaneous dose that would elicit the highest secretory response.

C. Maximal Subcutaneous Responses to Gastrin and Histamine

The relationship between the maximal subcutaneous responses to gastrin and histamine were studied in detail in 16 subjects.

Pattern of response to 40 ug/kg of histamine:

Despite the extensive use of histamine, the features of the secretory curve which follows administration of this stimulant are still/

TABLE A. Ten minute acid outputs (mEq) after subcutaneous administration of 40 ug. per kg. of histamine acid phosphate and 2 ug. per kg. of gastrin II.

No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	DU	GU	DU	DU	DU	N	GU	N	N	DU	GU	GU	GU	DU	N	PA
Sex	M	M	M	M	M	M	M	M	F	F	M	F	F	F	M	F
Age	54	44	42	51	30	30	59	35	46	23	53	26	54	55	30	67
Wt.(kg.)	77	77	77	84.5	72	84	62	68	60.5	48.5	62	44	56	53.5	82.5	71.5
	Histamine 40 ug./kg. s.c. Acid output mEq/10 mins.															
	4.20	5.13	1.08	4.18	3.00	2.00	3.27	1.55	0.90	2.60	0.40	2.31	0.26	0.82	-	-
	8.86	8.36	7.51	6.51	5.30	4.40	5.19	3.48	3.24	3.60	1.00	2.37	1.20	1.28	0.10	-
	10.22	9.51	7.56	6.90	7.14	6.83	5.58	4.26	3.41	3.50	2.34	2.80	1.71	1.52	0.43	-
	10.40	9.75	7.97	6.78	7.06	6.81	5.57	4.07	3.53	3.02	2.51	2.60	1.79	1.61	0.28	-
	10.32	8.46	5.45	6.16	7.00	6.98	5.58	4.16	3.43	2.70	2.95	1.79	1.69	1.48	0.17	-
	8.21	8.79	3.90	5.85	6.35	6.90	4.78	3.45	1.82	1.62	2.53	1.24	1.75	1.24	0.08	-
	8.67	8.47	3.00	3.81	5.59	5.78	3.55	2.41	1.29	0.97	2.41	1.24	1.51	1.22	-	-
Hour	52.21	50.00	33.47	36.38	35.85	33.92	29.97	20.97	16.33	17.04	11.73	13.11	8.40	7.95	1.06	-
Peak	61.88	57.78	46.08	41.04	42.40	41.28	33.46	24.98	20.74	20.24	15.98	16.20	10.41	9.22	2.13	-
	Gastrin 2 ug./kg. s.c. Acid output in mEq/10 mins.															
	5.02	4.95	0.27	3.23	4.73	1.72	2.38	0.81	0.63	0.94	0.72	0.12	0.44	0.44	-	-
	11.17	10.65	6.88	7.80	6.67	6.25	6.15	4.10	4.17	2.92	1.88	1.61	2.02	1.00	-	-
	11.39	11.49	9.31	8.32	7.93	8.06	7.79	5.46	4.30	4.46	4.06	2.61	1.61	1.49	0.07	-
	10.23	10.20	8.83	8.12	7.78	8.20	7.80	5.41	4.79	4.26	4.39	3.12	2.05	1.98	0.46	-
	10.23	10.20	8.83	7.95	7.92	8.20	7.29	4.63	4.00	3.86	3.99	3.86	2.56	1.95	0.67	-
	10.19	10.08	8.63	7.95	7.10	7.01	7.48	4.62	4.10	4.26	3.91	2.74	1.81	1.87	0.80	-
	10.14	9.58	7.99	6.51	6.40	7.50	7.05	4.25	3.81	3.71	3.76	2.71	2.24	1.87	0.88	-
Hour	58.23	57.57	42.75	43.37	42.13	39.44	38.89	25.03	21.99	20.70	18.95	14.06	10.49	8.73	2.00	-
Peak	67.68	66.42	53.40	48.51	47.26	48.92	46.77	32.61	27.27	26.16	25.35	20.94	13.83	11.51	5.04	-

TABLE B. Ten minute volumes following subcutaneous administration of 40 ug. per kg. of histamine acid phosphate and 2 ug. per kg. of gastrin II.

No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	DU	GU	DU	DU	DU	N	GU	N	N	DU	GU	GU	GU	DU	N	PA	
Sex	M	M	M	M	M	M	M	M	F	F	M	F	F	F	M	F	
Age	54	44	42	51	30	30	59	35	46	23	53	26	54	55	30	67	
Wt.(kg.)	77	77	77	84.5	72	84	62	68	60.5	48.5	62	44	56	53.5	82.5	71.5	
	Histamine 40 ug/kg. s.c. Vol. mls./10 mins.																
	49	57	28.5	38	38	20	39	18	12	33	13	59	12	22	9.5	-	
	71	74	74	53	45.5	31	45	28	27.5	34	29	29	17	19.5	9.5	-	
	83	78	74	52	59.5	48	45.5	31	26	32	32	31.5	18	19	10.2	-	
	83	78	79	60	56.5	48	42	29	25	28	32	25.5	16.5	21.5	6.8	-	
	74	73	53	48	60.5	49	43	29.5	25	26	34	18.5	17.5	20	8.5	-	
	69	73	54	47	52.5	47	39.5	27	14.5	18.5	32.5	13.5	17.5	18	9	-	
	68	74	40	33	45.5	40	32.5	25	12	14.5	34	12	15	17	6	-	
						17											
Hour	429	433	362	298	312	243	254	162	130	171	172	177	99	120	54	-	
Peak	498	450	454	330	353	288	263	176	155	191	197	172	104	122	58	-	
	Gastrin 2 ug/kg. s.c. Vol. mls./10 mins.																
	54	66	18	39	52	21	33	13	8.5	18	15	25	12	21	9.5	-	
	88	96	85	64	54.5	53	48	46	32	36	32	31	18	25	15.5	-	
	87	97	90	65	64.5	62.5	59.5	42	31	46	41	31.5	18	28	13	-	
	75	85	90	64	62.5	61	58.5	42	35	42	43	34	17.5	26.5	18.5	-	
	80	81	89	62	65.5	61	54	35	29	37.5	43	46	20	24	20.5	-	
	79	75	84	58	56	56	57	35	30	42	42	33.5	20	21.5	20	-	
	76	71	74	53	56	56	57	32	30	39.5	38	33.5	20	24	26	-	
	83	69	68	54	53		54	30		40	36	39		16.5	20	-	
	73	71	68	54	54		50	25		32	37	43.5		19	13.5	-	
	72	66	61	50	54		52	23		33.5	36	36		18	15	-	
	70	64	64	48	52		50	23		33	36	33		20	16	-	
	70	67	49	52	52		43	20		33.5	36	43.5		15	14.5	-	
Hour	463	500	456	352	355	315	310	213	165	222	216	201	106	146	97	-	
Peak	525	579	526	384	384	370	340	260	187	247	254	219	114	155	126	-	

TABLE IV. Means of the data from Tables A and B:
gastrin 2 ug/kg (left), histamine 40 ug/kg (right).

10-minute period.	Volume mls.	H^+ mEq/L	Acid output mEq.	Volume mls.	H^+ mEq/L	Acid output mEq.
1	32.2	63.2	2.03	36.3	70.0	2.54
2	55.6	99.8	5.55	45.1	107.0	4.83
3	60.7	112.7	6.84	50.0	113.6	5.68
4	57.9	116.2	6.72	49.1	116.7	5.73
5	57.9	114.3	6.62	44.4	118.5	5.26
6	55.4	116.2	6.43	42.2	108.5	4.57
7	52.2	112.5	5.88	33.6	116.9	3.92
8	50.0	113.9	5.69			
9	47.7	112.3	5.35			
10	45.7	114.7	5.25			
11	44.9	114.0	5.12			
12	43.4	109.4	4.75			

— The peaks of volume and acid outputs for histamine and gastrin from which the peak hour output is calculated occur in the second 20-minute period (periods 3 and 4).

— The ratio of the post-stimulatory to peak hours is 83.6% for histamine and 84% for gastrin.

— The ratio of the output of histamine to that of gastrin is 83.7% for the first hour and 84% for the peak hour.

still the subject of much controversy. Although the main discussion of this problem is deferred to the section on the kinetics of secretion, the following observations may be made.

The onset of secretion following histamine is rapid. The volume and concentration of the first 5-minute specimens are invariably higher than the corresponding basal specimens, and the long latency to this stimulant described in animals is not a feature of its action in man. Peak volume and acid output are attained simultaneously in the second 20-minute period following stimulation, and the decline of secretion from this level is fairly rapid (Fig. 5 and Tables A and B).

Pattern of response to 2 ug/kg of gastrin:

As with histamine, the onset and rise of secretion following this dose of gastrin are rapid. The peaks of volume and acid outputs are again clearly evident in the second 20-minute period following stimulation (Figs. 5 and 6). The decline of secretion, however, is more gradual than with histamine. The output at the end of two hours is usually around 75% of peak response and stimulated secretion lasts for up to 3 to 4 hours.

The responses obtained from 16 normal and ulcer subjects are listed in Tables A and B. The series is recorded in declining order of magnitude from left to right. The acid output was calculated in the two ways already described and the values are recorded for/

for the post-stimulatory and peak hours for each subject following gastrin and histamine. Table IV from which Fig. 5 was plotted gives the means of acid output, secretory rate and concentration per 10 minutes for this series. Subject 16, a proved case of pernicious anaemia, responded to gastrin by producing a few millilitres of an alkaline fluid with high sodium concentration.

Discussion

It is clear from the data obtained in this study that subcutaneous stimulation by histamine in a dose of 40 ug/kg does not elicit the maximal response of which the human stomach is capable. This is confirmed by the work of Lawrie et al.[67] on the intravenous infusion of this stimulant. What 40 ug/kg represent is the maximal effective dose of histamine when delivered by subcutaneous injection[68]. The same conclusion applies to gastrin delivered by subcutaneous injection in a dose of 2 ug/kg.

Fig. 7 shows the relationship between the acid output for gastrin and histamine during the hour following stimulation. Fig. 8 shows the relationship between the peak hour outputs following both stimulants. The linear regression functions obtained from the data in Figs. 7 and 8 are closely similar, and a combined regression using the post-stimulatory and peak hour values is shown in Fig. 9. A similar combined regression for secretory rate (volume output) is shown in Fig. 10.

Inspection/

Inspection of the linear regressions in Figs. 9 and 10 shows the close correlation between the variables. From this finding two inferences may be drawn: (i) that a particular stomach responded in a closely similar way on the different occasions it was examined, and (ii) that there are no notable differences between individuals in the way the stimulants elicit secretion.

That the maximal concentration achieved under both modes of stimulation must be virtually the same is confirmed by comparing the slope relating secretory rates from the two stimulants with the slope relating acid outputs. This is further confirmed by inspection of the mean concentration values in Table IV. This conclusion is only of general validity since salivary contamination in this study may have masked a slight tendency to higher concentration in the histamine samples (see chapter on electrolytes).

The relative effectiveness of gastrin as a secretory stimulant compared to histamine can be judged from Figs. 5 and 9, when it will be seen that gastrin produces an appreciably greater response from the stomach of the order of more than 10%. The correlation between these two methods of stimulating secretion, however, is highly significant. This appears to mean that all the data obtained in the past with histamine in maximal subcutaneous doses are still valid, and in no way outdated by the more potent gastrin. If the effectiveness, in terms of equal response of gastrin to histamine base, are compared weight for weight, it seems that gastrin is some thirty times more effective. If, however, the comparison is made

on/

on a molar basis, then it seems that gastrin is some five hundred times more effective than histamine.

As a secretory stimulant, gastrin has clear advantages over histamine combined with an anti-histamine, since only one injection is necessary, no side-effects have been observed, and the soporific effect of the anti-histamine is avoided. Unless abnormal secretory conditions are encountered where histamine and gastrin fail to produce roughly equivalent effects, so that it becomes necessary to give both stimulants, the use of histamine with an anti-histamine as a secretory stimulant is likely to be abandonned if gastrin or one of its more potent analogues becomes readily available commercially. In two years' experience with the stimulant, and in the present series of normal and ulcer subjects, no such divergent action has been encountered.

If gastrin is used as a secretory stimulant, then subcutaneous administration provides in the post-stimulatory and peak hour outputs accurate measures of gastric secretory activity.

D. Kinetic and Dose-response relationships following Subcutaneous Administration of Stimulants

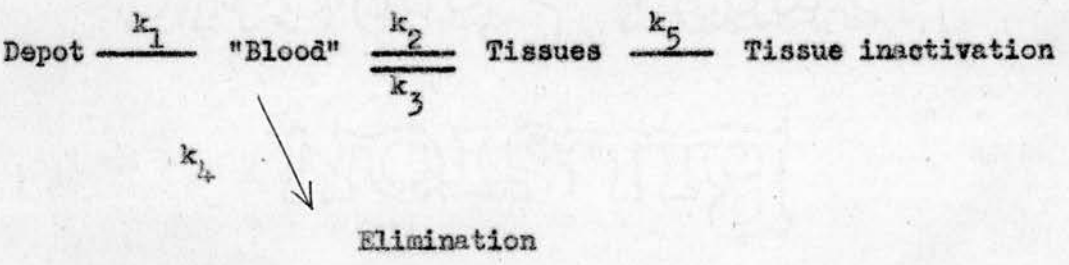
The purpose of secretory tests is to provide a stable and discriminating measure of the functional capacity of the stomach. The emphasis in this statement is on the ability of a secretory test to provide a repeatable index rather than an absolute measure of the utmost capacity of the stomach to secrete. Before this problem

can/

can be resolved and the numerous misconceptions surrounding it dissipated, attention must be paid to the kinetics, or time-output relationships, of secretion.

The determinants of peak response:

According to Teorell [59], the passage of a drug across tissue boundaries may be regarded as occurring in a series of consecutive processes obeying Fick's law of molecular diffusion. The scheme of distribution may be described as follows:



All these processes are controlled by rate determining constants, k_1 to k_5. Thus the passage of a drug from depot to blood (blood being taken here to include the volume of interstitial fluid) is a unidirectional process dependent on the resorption rate. Inactivation in the tissues and elimination by the kidneys are irreversible processes determined by the prevailing concentrations in the tissues and blood respectively. Blood-tissue passage is bidirectional and determined by the prevailing concentration difference in these two compartments.

Following subcutaneous administration, the drug accumulates rapidly/

rapidly in the blood during a short initial period. The concentration soon reaches a maximum and decreases rather slowly and almost exponentially. Maximal tissue concentration is distinctly lower and is attained at a much later time. As a rule, the slower the resorption rate (k_1 diminished), the lower the concentration in the blood and tissues and the later the concentration maximum appears. The exact mathematical relations are not easy to express for the general case, but may be obtained clearly cut for somewhat simplified conditions. The equations resulting from this treatment are as follows:

$$t_{max} = \frac{2.3}{k_1 - k_4} \log \frac{k_1}{k_4} \qquad \text{Eq. 1}$$

$$y_{max} = D \cdot \frac{k_1}{k_4} \frac{k_4}{k_4 - k_1} \qquad \text{Eq. 2}$$

where D represents the subcutaneous dose, and t max and y max, the time of appearance and the height of the maximal blood concentration respectively.

It is clear from Eq. 1 that the time of appearance of maximal concentration is independent of the dose, but conditioned by the rates of resorption (k_1) and elimination (k_4). Any diminution in the resorption rate causes the maximum to appear later.

From Eq. 2, it may be deduced that the maximal concentration is directly proportional to the injected dose and approximately directly/

directly proportional to the resorption intensity and inversely to the elimination intensity. These conclusions may be extended to the tissue conditions the more rapidly the tissues take up the drug. It will be recalled that similar conclusions had been arrived at for the case of intravenous administration of a stimulant and their validity attested by the good fit of the secretory data.

The pattern of secretory response follows closely the pattern of distribution of the stimulant in the tissues. Following subcutaneous injection of a stimulant, one of the essential factors which determine the shape of the tissue concentration curves is the rate of resorption. This factor, which is absent in the case of intravenous injection, is not always easy to control (siting of the injection, degree of adiposity, intensity of local blood flow), and engenders fluctuations in the shape of the secretory curves. The experimental data, however, show that the general validity of the conclusions from Eqs. 1 and 2 is unimpaired. Thus, the time of appearance of peak output was in the second 20-minute period following subcutaneous administration of 1,2,3 or 4 ug/kg of gastrin. It was mentioned earlier that peak output following administration of 0.5 ug/kg of gastrin tended to occur some ten minutes later than with the higher doses. Increased ionisation which accompanies the lowest dilutions of this stimulant may affect the resorption intensity and explain the observed delay.

The delay in the appearance of peak response following administration/

administration of even large doses of histalog may well be related to the intensity of its absorption and elimination.

The data obtained by Marks, Komarov and Shay [63,64] in dogs confirm the findings in man. Despite a 64-fold increase in the dose of subcutaneous histamine, the time of appearance of peak output was virtually unchanged.

The data from the same authors [63,64] further confirm the conclusions derived from Eq. 2, namely, the relationship between peak output and the subcutaneous dose of the stimulant. In this study on man, no attempt was made to obtain a precise relationship between peak output and the subcutaneous dose of gastrin. More emphasis was placed on establishing the dose of gastrin that would elicit the highest possible response from the stomach.

The output expected from intravenous injection of 2 ug/kg is around 96 to 98% of the calculated maximal output (Fig. 6, Ch. VI).

The peak response to subcutaneous injection of 2 ug/kg of gastrin in subjects GM and JM is between 90 and 92%. It is doubtful whether, at this level, even a substantial increase in the subcutaneous dose would result in more than a fractional increase in output. In fact, subcutaneous administration of 3 to 4 ug/kg resulted in an output which was virtually indistinguishable from that following 2 ug/kg. In this sense, 2 ug/kg could be defined as the maximal subcutaneous dose of gastrin, and the peak output which it elicits as the maximal subcutaneous response. This response, close on 90%, can, thus, be considered a reliable and stable/

stable function of the maximal capacity of the stomach to secrete in response to exogenous gastrin. If the peak response of various subjects to 2 ug/kg of gastrin is taken as 90% and their peak responses to various doses calculated proportionately, a fairly good subcutaneous dose-response curve may be obtained (Fig. 11).

It is clear from the foregoing that the three features which describe a secretory peak are its time of appearance, its magnitude and its duration. The duration of peak response varies with the mode of administration and is longer with continuous infusion (20 to 40 minutes) and subcutaneous injection (20 minutes) than with prompt intravenous injection (10 minutes).

While all three features may be influenced by variations in the resorption rate, the main source of error in the estimation of a secretory peak arises from inaccurate collection. For example, it was noted above that peak response occurred during the second 20-minute period following stimulation by 2 ug/kg of gastrin or 40 ug/kg of histamine. On examination of the data from 45 gastrin tests and 29 histamine tests, it was seen that only 80% of the cases in each series showed a peak output in the second 20-minute period. The remaining 20% showed a peak output in the third 20-minute period, but the excess output in this period over the second was never more than 0.5 mEq for histamine and 1 mEq for gastrin and usually much less. On closer inspection of the five-minute volume outputs in these cases, it was clear that an error of collection was responsible for the discrepancy. Errors of collection/

collection tend to have a sequence in which undercollection is followed by overcollection. If 0.5 mEq is taken arbitrarily as the limit of error that may be attributed to miscollection, then all histamine tests and all but three of the gastrin tests show a peak output in the second 20-minute period.

Besides altering the timing of a peak, errors of collection produce a spurious extension of its duration and lead to an underestimate of its magnitude. That is why the key to accurate measurement of peak response as an index of gastric secretory activity lies in the frequent sampling of the juice.

Alternative measures of secretion:

A number of alternative measures of gastric secretory activity have been proposed over the last fifteen years by various workers. Kay [68] measured maximal response to histamine between the 15th and the 45th minute following stimulation. Baron [69,70], who noted considerable fluctuations in the timing of the peak when 15-minute collections were used, took the sum of the two highest consecutive 15-minute outputs as indicating "peak acid output" (P.A.O.). Lawrie et al. [67] introduced histamine by continuous intravenous infusion and elicited a higher response than with subcutaneous injection. Card and Marks [62] collected the juice over the whole post-stimulatory hour and devised the "maximal acid output" or M.A.O. as the measure most likely to minimise errors of collection.

The theoretical foundations for all these measures have been lacking/

TABLE V. Correlation coefficients for the regression of the outputs in one 10-minute period on the outputs in the subsequent 10-minute period.

	Correlation coefficients	
10-minute period	Gastrin	Histamine
1st versus 2nd	0.854	0.827
2nd versus 3rd	0.973	0.972
3rd versus 4th	0.998	0.997
4th versus 5th	0.990	0.971
5th versus 6th	0.987	0.948
6th versus 7th	0.989	0.950

lacking and their validity rests, in the main, on statistical evidence. Further analysis of the kinetics of secretion, however, discloses a number of important features and displays the inter-relationships of these various measures.

It will be observed that all these measures have been derived from various components of the secretory curve. The question that must be asked is to what extent these components, as represented by the successive periods, are related to each other, to the peak response, and to the output of the whole hour. It may, for example, be asked whether the secretory curve in Fig. 5, which is derived from the mean outputs of a series of 16 subjects (Table A), is a representative curve, or whether some subjects show notable deviations from its course.

The relationships of the outputs from successive 10-minute periods following histamine and gastrin are portrayed in a series of plots in Figs. 12 and 13. The 45 degress line is drawn for purposes of visual clarity and the progress of the slope with respect to it indicates the rise and decline of secretion. Table V gives a summary of the results. It is clear from the highly significant correlations obtained that the secretory curves, and hence the patterns of distribution and metabolism of the stimulant, in different individuals, are closely similar.

The inference from these findings is that all measures of secretion derived by summation or other manipulations of the successive collection periods may be considered as related indices of secretion provided it is clearly recognised that they are functions/

functions and not absolute measures of gastric secretory capacity. The value of any single measure will approach the value of peak response depending on which segment of the secretory curve it was calculated from.

Unlike peak response, which because of its high level is the least variable element of the curve, the other measures, derived in part from either side of the peak, are subject to minor variations from fluctuations in the background vagal tone or the rate of resorption of the stimulant. The influence of this latter factor is seen in the somewhat greater degree of variability in the output of the first 10 minutes following stimulation (Figs. 12 and 13). The influence of background vagal tone is most evident during the second hour following stimulation by gastrin. The upper curve in Fig. 14 is derived from the outputs of normal and ulcer subjects tested for the first time and in whom, it has often been noticed, spontaneous secretion was high. The lower curve is derived from the outputs of subjects JM and GM in whom spontaneous secretion was either absent or reduced to very low levels. Administration of mechothane to subjects JM and GM had no effect on the peak response during the first hour, but tended to shift the shape of the curve upwards during the second hour to approximately the same level as the upper curve. It is probable that the influence of a higher background vagal tone in normal and ulcer subjects tested for the first time is responsible for keeping the response to the falling

level/

0 - 10		10 - 20		20 - 30		30 - 40		40 - 50		50 - 60		60 - 70		70 - 80		80 - 90	
H	P	H	P	H	P	H	P	H	P	H	P	H	P	H	P	H	P
8.7	11.5	17.0	20.2	20.7	26.2	31.6	36.5	41.2	46.5	50.0	57.8	62.0	74.7	75.4	80.7	81.9	91.1
8.0	9.2	———		21.5	26.9	33.7	46.1	42.1	51.5	50.1	56.9	62.4	76.2	77.5	94.7	83.6	95.7
———		10.5	13.8	25	34.9	35.8	42.4	42.1	47.3	52.0	63.1	62.9	67.8	78.4	98.4	———	
8.4	10.4	11.7	16.0	———		36.4	41.0	42.6	53.2	52.2	61.9	———		———			
———		13.1	16.2	22.0	29.4	39.9	44.2	42.7	53.4	54.4	62.1						
1.1	2.1	14.1	20.9	26.6	29.0	———		43.4	48.5	57.6	66.4						
2.0	5.0	19.0	25.3	———		30.0	33.5	44.5	53.0	58.2	67.7						
		19.0	25.3	20.0	22.8	34.0	44.6	45.2	53.7	———							
		19.8	31.6	21.0	25.0	38.9	46.8	45.4	52.8	54.8	60.3						
		———		21.3	24.6	———		48.6	56.1	50.3	54.6						
		16.3	20.7	22.0	27.3	30.3	35.1	48.6	57.2								
		17.9	23.7	25.	32.6	30.6	37.4	49.3	54.6								
						32.3	35.5	———									
						33.9	41.3	45.1	52.6								
						34.9	39.6										
						36.4	53.4										
						36.8	48.1										
						38.8	42.9										
MEAN						39.4	48.9										

D.U.	8.3	10.3	17.0	20.2	22.4	29.3	35.5	42.0	44.6	52.3	53.5	62.3	62.4	72.9	77.1	91.3	82.7	93.4
G.U.	8.4	10.4	15.3	21.3	24.3	29.2	34.3	41.6	45.1	52.6	-	-	-	-	-	-	-	-
N	1.5	3.5	17.1	22.2	21.9	26.5	34.8	42.5	-	-	52.5	57.4	-	-	-	-	-	-
APP	5.6	7.6	15.8	21.4	22.5	27.9	34.8	42.0	44.7	52.3	53.3	61.2	62.4	72.9	77.1	91.3	82.7	93.4

TABLE VI. The hour (H) and peak (P) outputs from all the gastrin and histamine tests. Points above the red line are from D.U. patients, and points above the green line are from G.U. patients.

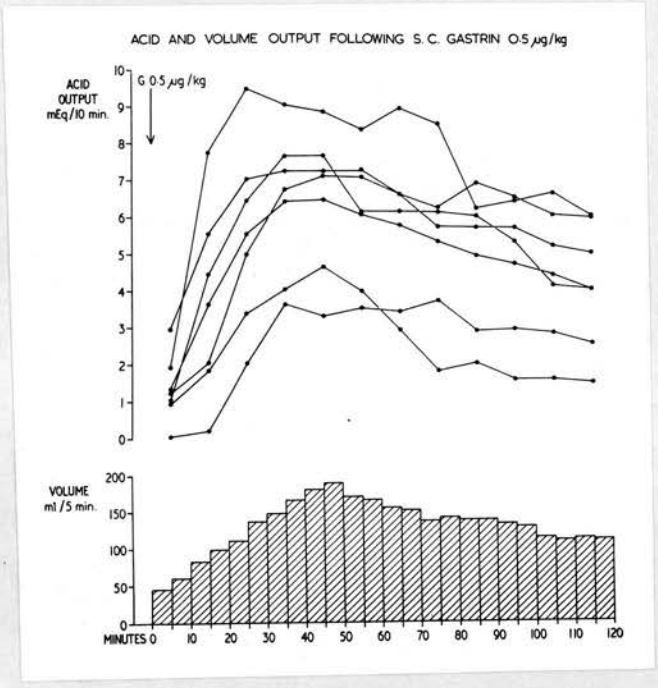
level of gastrin during the second hour at a high level. This is further revealed by the striking linear relationship between the responses of the first and second hours, $r = 0.994$, $n = 13$ (Fig 14).

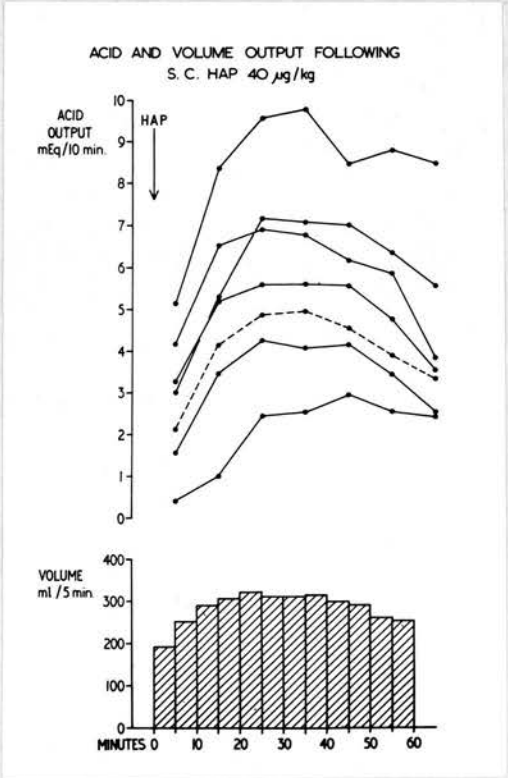
If the value of peak response as calculated from the second 20-minute period is compared with the value of the post-stimulatory hour following gastrin and histamine, a highly significant correlation is obtained (Fig. 15, Table VI). The interest of this finding is that it provides an important link between theoretical foundation and experimental fact. The significance of peak or maximal subcutaneous response is that it is a stable and close function of the calculated maximal response, which in turn is dependent on activation of all the secretory units in the stomach. The significance of the post-stimulatory hour response, in this context, is that it has been shown by Card and Marks [62] to correlate closely with the parietal cell population of the stomach. Thus the conclusions of the preceding chapters are rejoined and confirmed, that the final discriminating parameter between individuals is the size of their parietal cell mass as manifested in their maximal acid outputs. Despite the overlap which is expected from the normal spread in a population, the only feature that appears to distinguish duodenal ulcer patients is the larger size of their stomachs (Table VI).

If the means of decades of acid output are plotted against the means of the corresponding body weights, a highly significant linear correlation is obtained (Fig. 16). The inference from this is/

is that the parietal cell mass is closely related to the total body cell mass, and that any simple and accurate measure of body cell mass may prove to be a reliable index of gastric activity.

If what distinguishes a duodenal ulcer patient is merely a more than average weight, the question may justifiably be asked whether duodenal ulceration is simply a manifestation of disordered growth.





Figures 1 and 3: The patterns of response in six subjects to subcutaneous administration of 0.5 ug/kg of gastrin and 40 ug/kg of histamine.

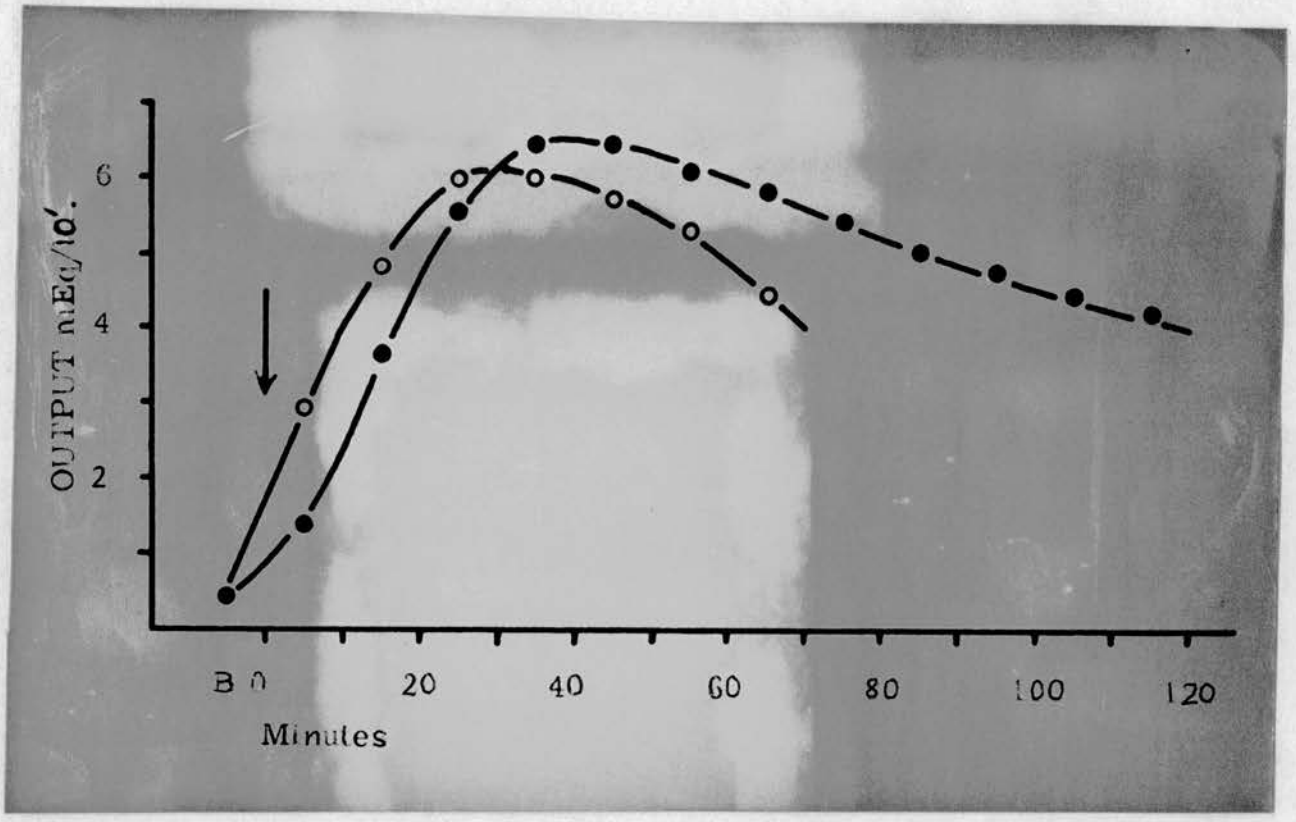


Figure 2: Mean acid output per 10-minute period following subcutaneous administration of 0.5 ug/kg of gastrin (closed circles) and 40 ug/kg of histamine (open circles) in six subjects. Data from Table II.

SUBJECT	DOSE	OUTPUT m. Eq. Post-gastrin hour.	OUTPUT m. Eq. Peak period.
A. NORMAL (64 kg.)	½ ug/kg s. c.	19	26
	2 ug/kg s. c.	25.8	32.8
	4 ug/kg s. c.	25	32.6
B. NORMAL (84. kg.)	1 ug/kg s. c.	29.5	42.3
	2 ug/kg s.c.	40.2	50.4
	Maximal infusion I. V.	41.1	51.4
	I. M. 1 ug/kg	38. 2	49.0
	I. M. 1.5 ug/kg	38.0	48.5
C. D.U. (77.5 kg.)	2 ug/kg	57.2	66
	3 ug/kg	58.2	67.5

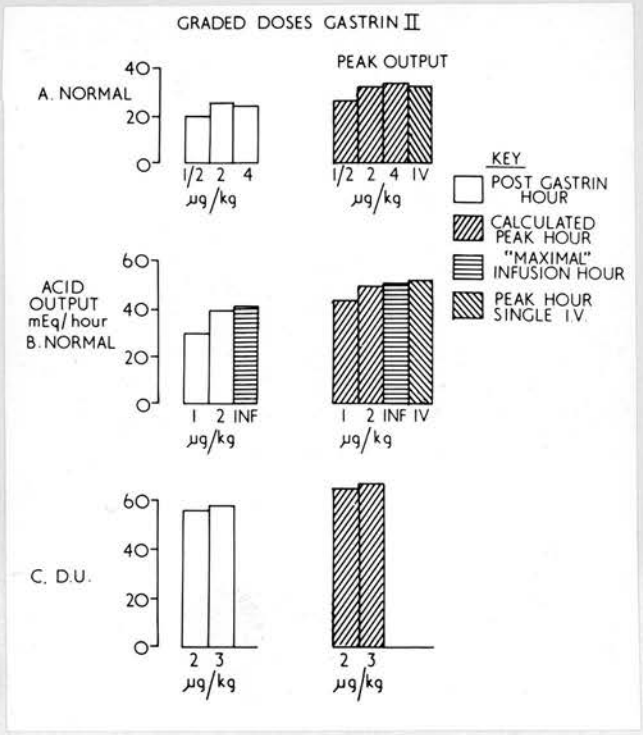
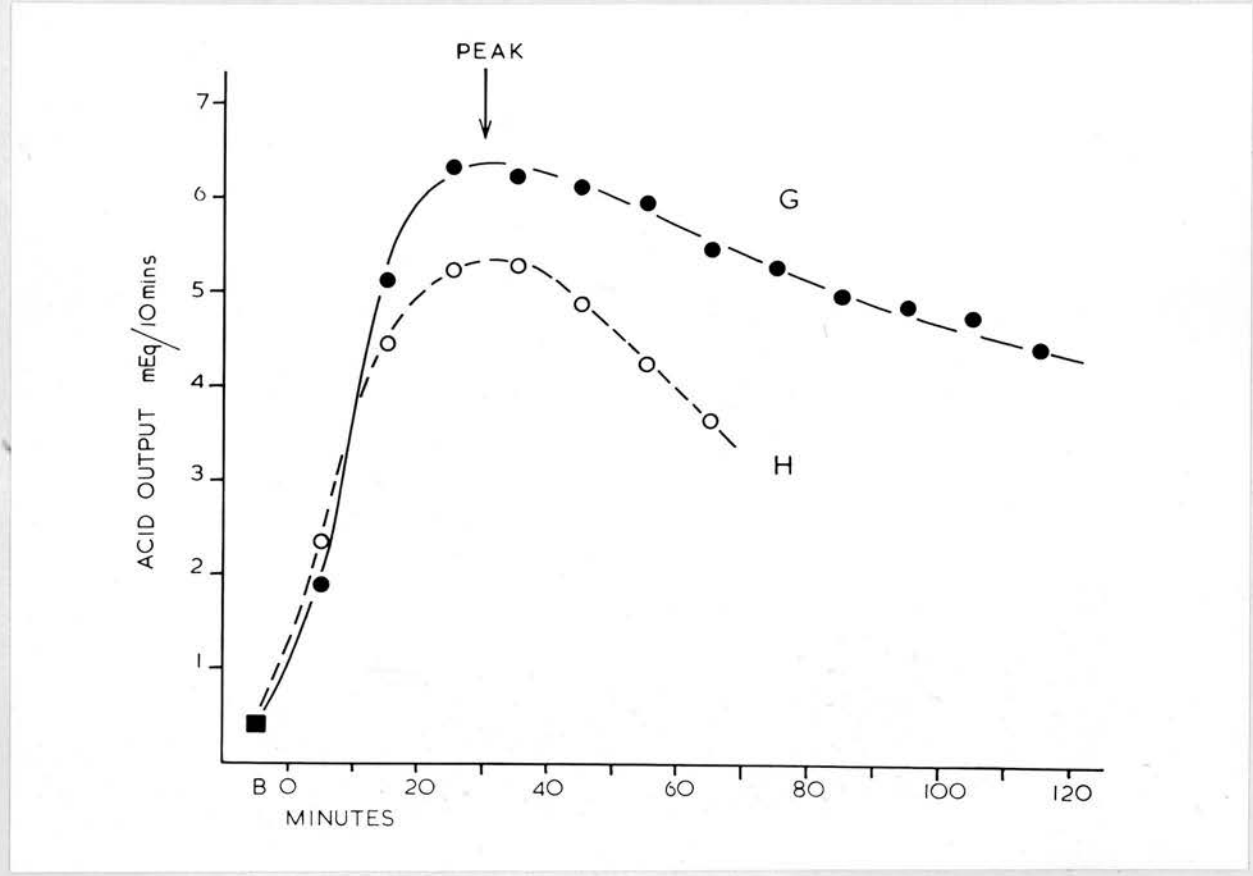


Table III, Figure 4: The responses of 3 subjects to increasing doses of gastrin.



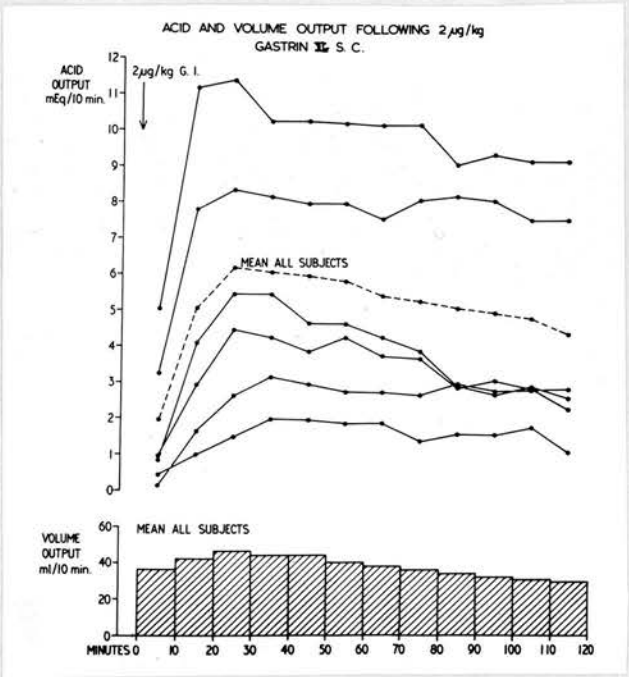
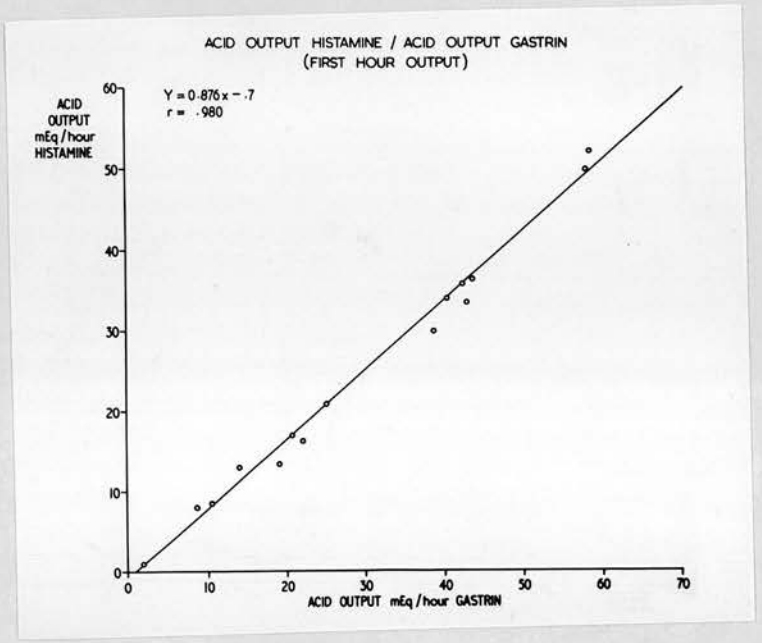


Figure 5: (above) Mean acid output per 10-minute period following subcutaneous administration of 2 ug/kg of gastrin (closed circles) and 40 ug/kg of histamine in 16 subjects. Data from Table IV.

Figure 6: (below) Representative responses following 2 ug/kg of gastrin (taken from Tables A and B).



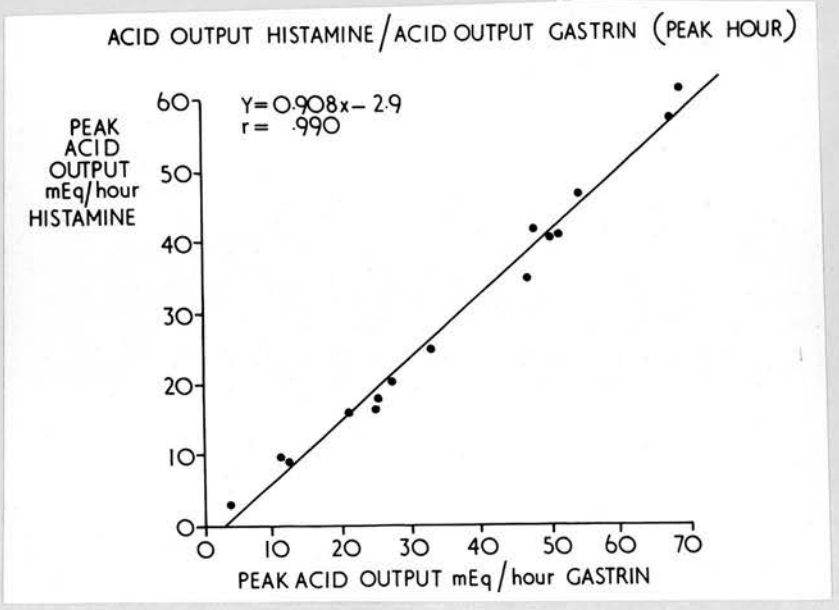
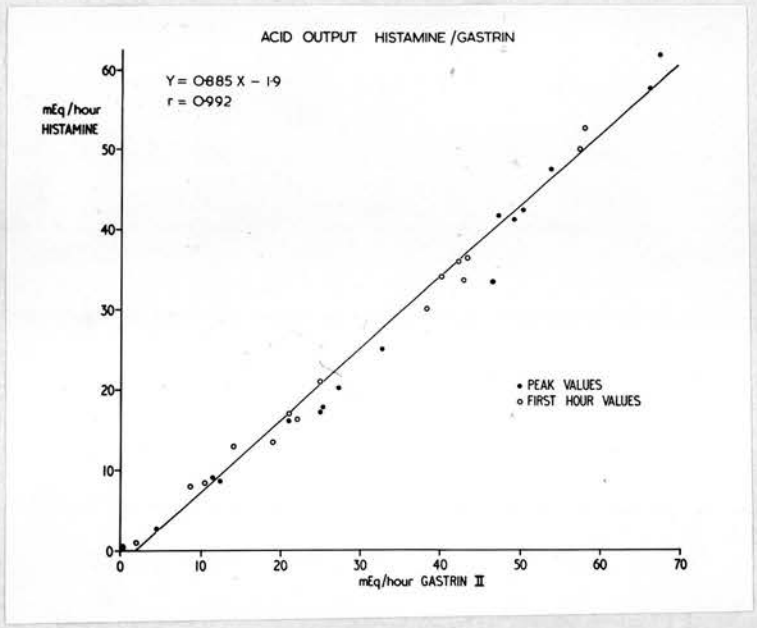


Figure 7: (above) The relationship between the post-stimulatory hour outputs of gastrin and histamine.

Figure 8: (below) The relationship between the peak hour outputs of histamine and gastrin.



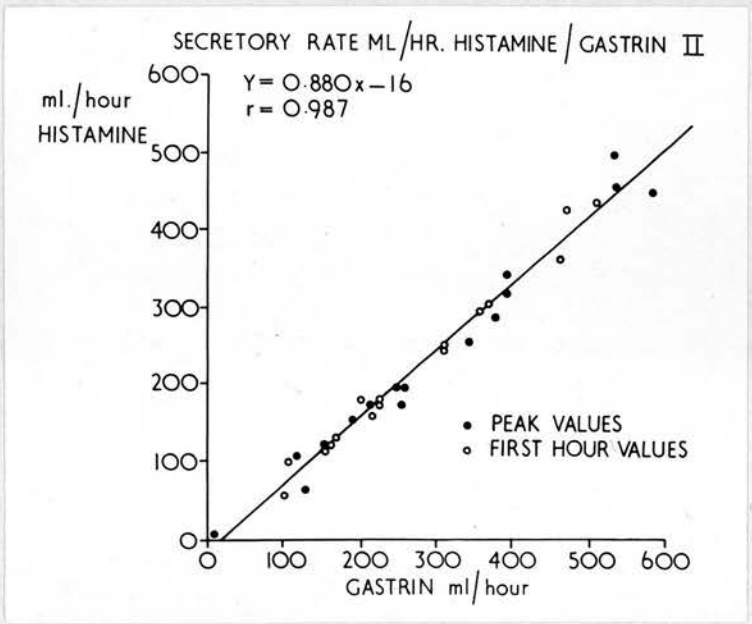


Figure 9: (above) Combined regression of the post-stimulatory and peak hour acid outputs for histamine on the corresponding values for gastrin. Data from Table A.

Figure 10: (Below) Combined regression of the post-stimulatory and peak hour volume outputs for histamine on the corresponding values for gastrin. Data from Table B.

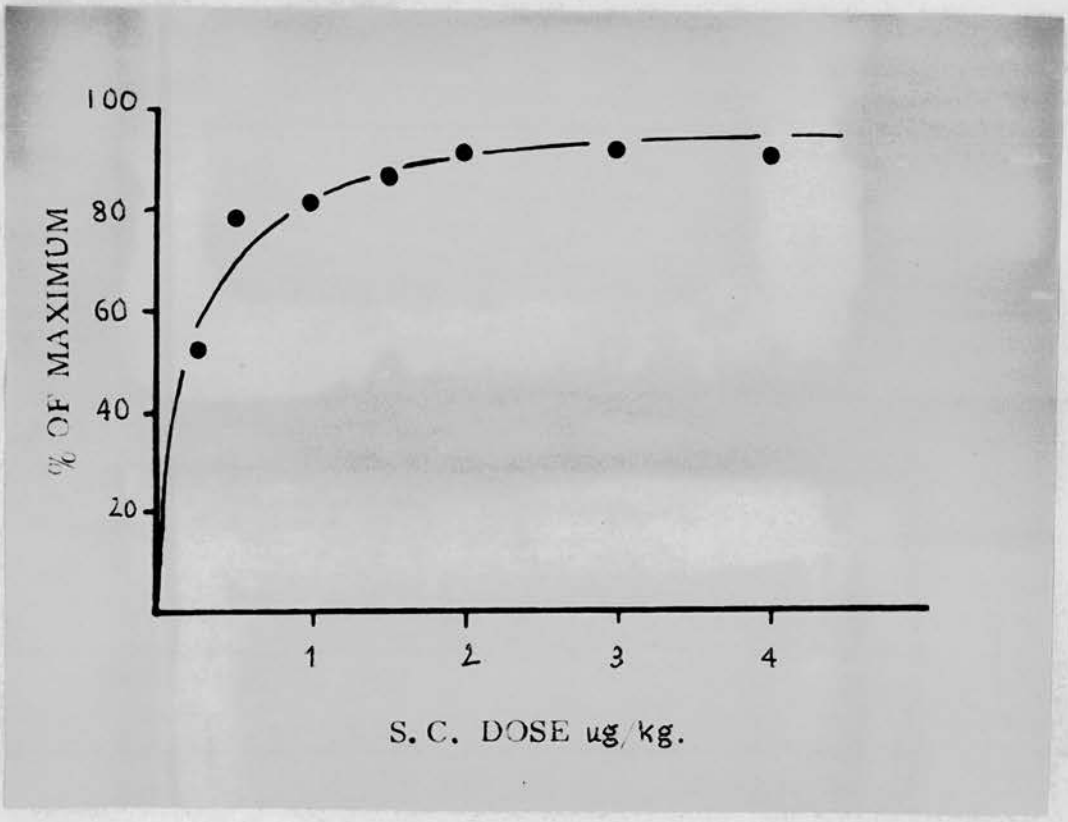


Figure 11: Subcutaneous dose-response curve. The peak responses from various subjects are expressed as percentages of their maximal response.

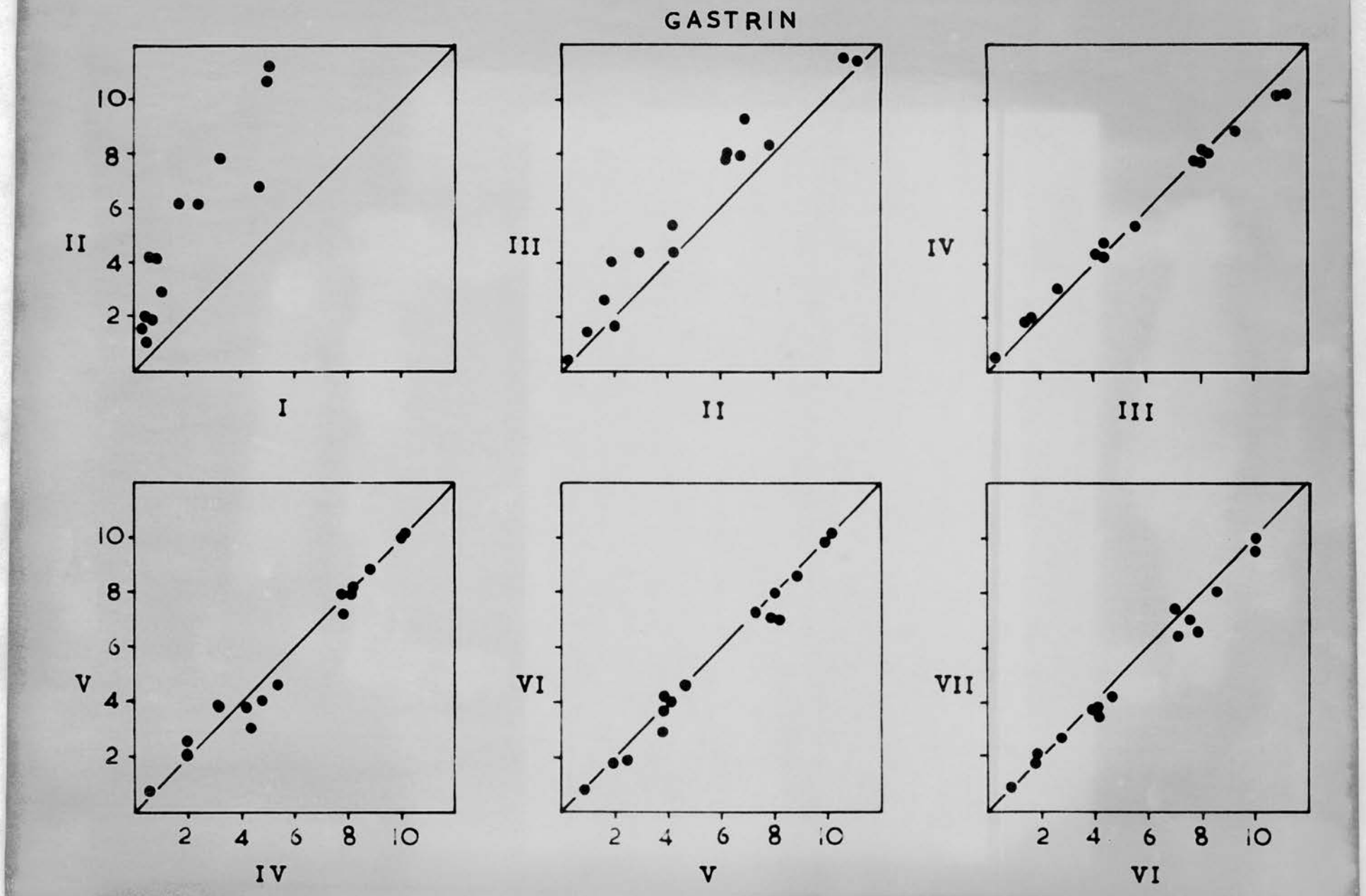


Figure 12: The acid outputs in a ten-minute period plotted against the outputs in the subsequent period. The diagonal is drawn at 45 degrees. The equality between the outputs of the third and fourth ten-minute periods is apparent. Data from Table A.

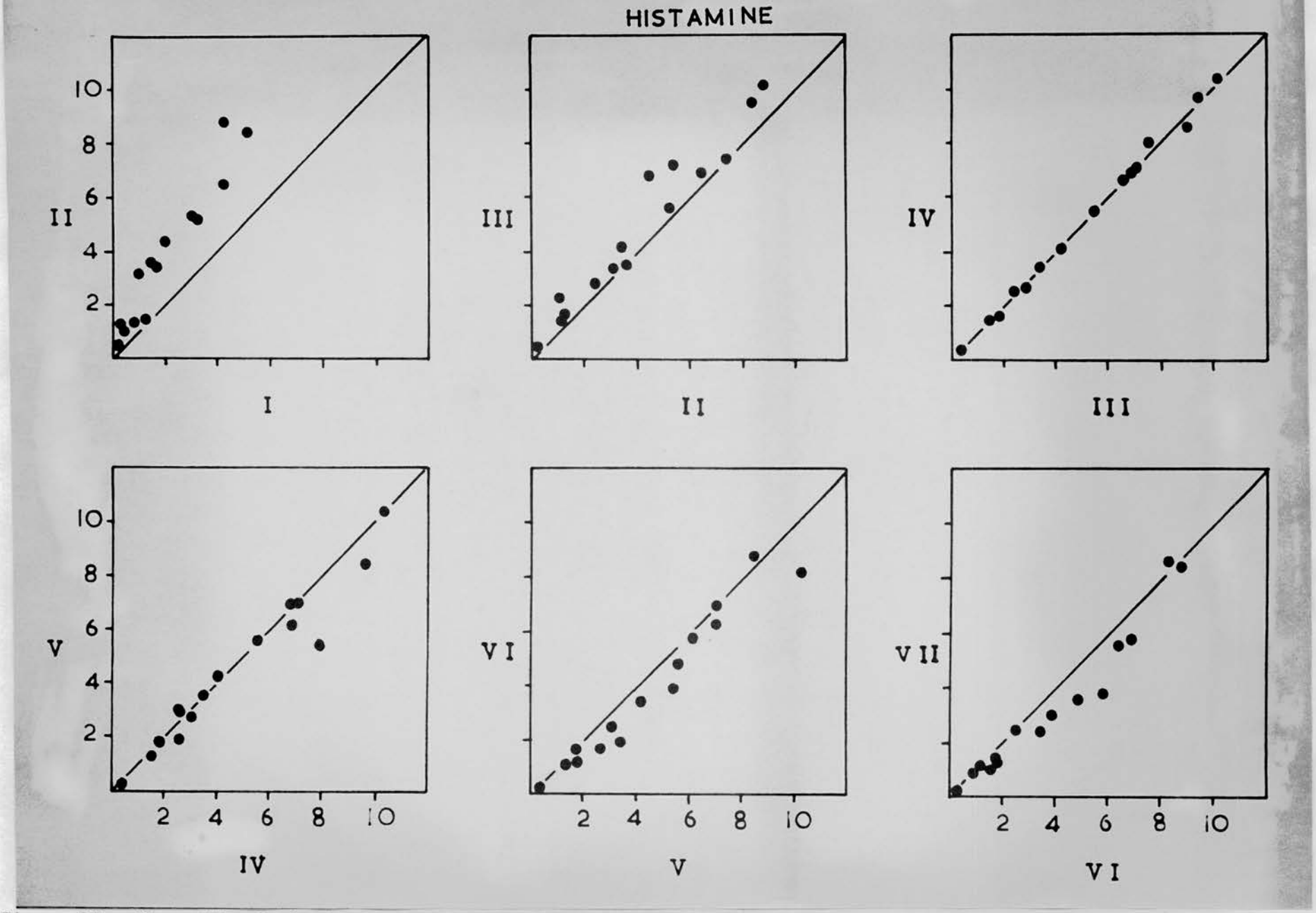
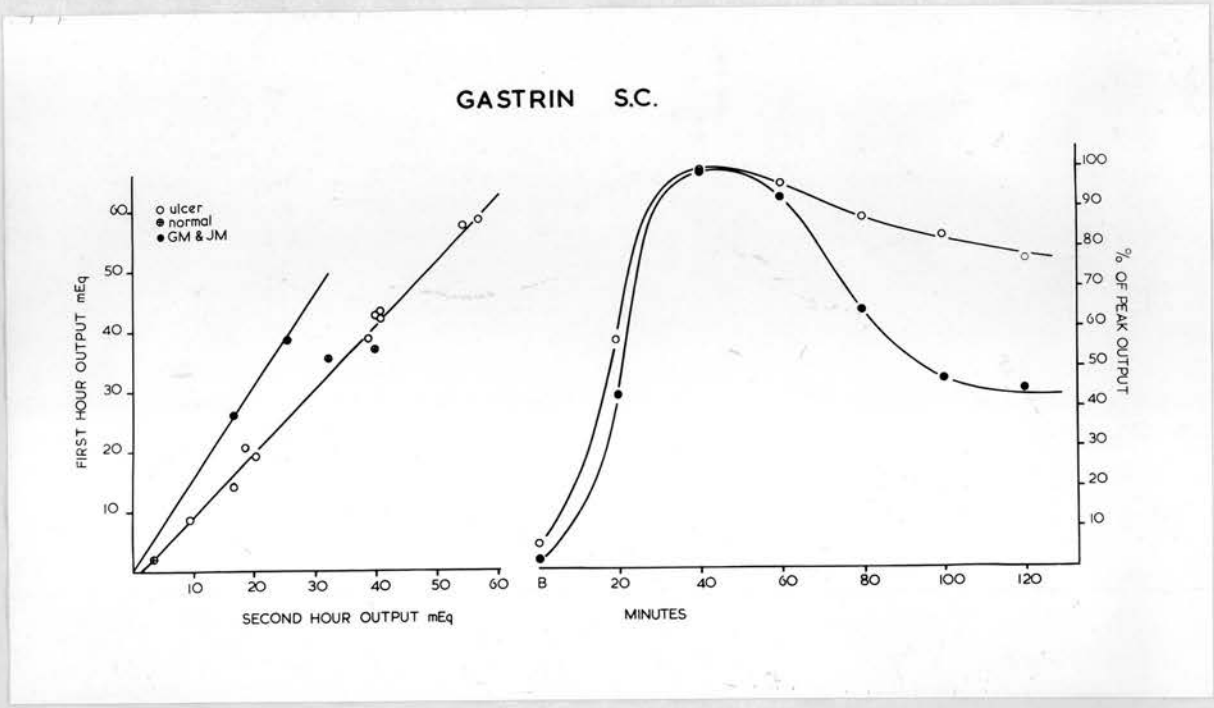


Figure 13: The acid outputs in a ten-minute period plotted against the outputs in the subsequent period. The diagonal is drawn at 45 degrees. The equality between the outputs of the third and fourth periods is apparent. Data from Table A.



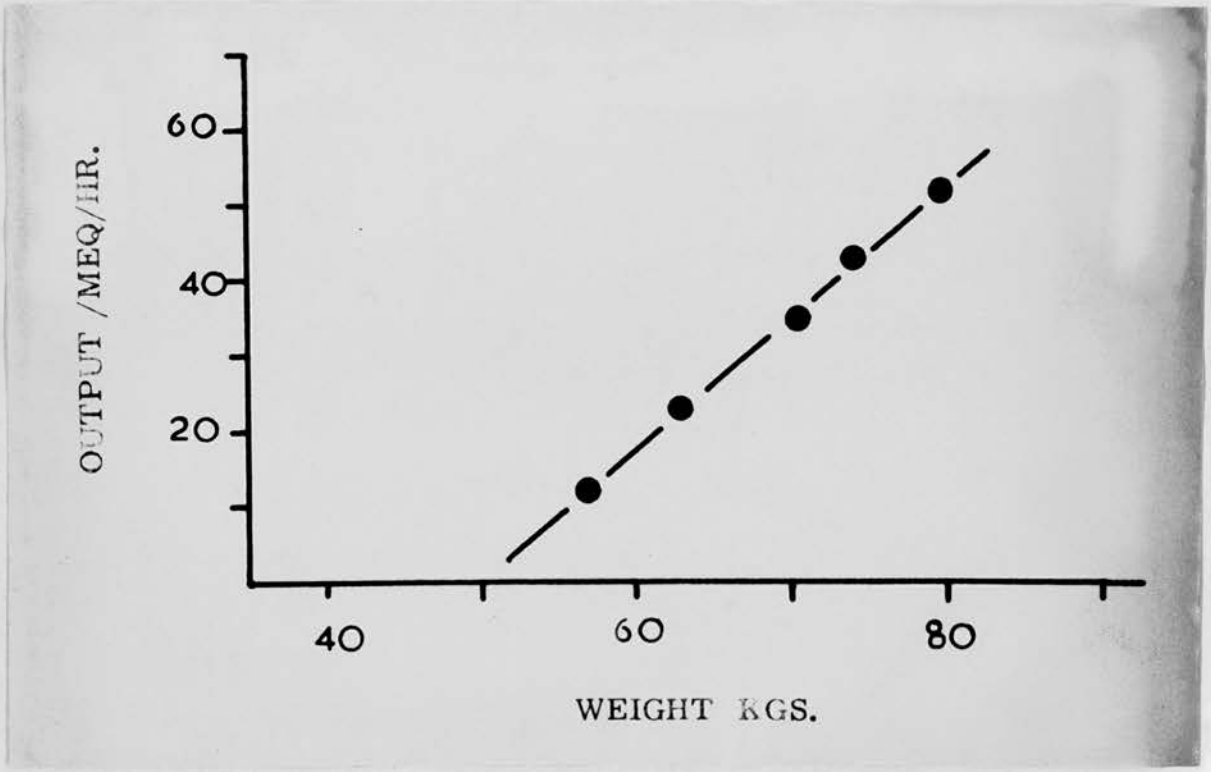


Figure 14: (above) The 20-minute outputs expressed as percentages of the respective peak outputs (i) from subjects GM and JM (closed circles) and (ii) from 13 ulcer and normal subjects tested for the first time (open circles). The plot on the left side shows the relationship between the first and second hours in the two groups.

Figure 16: (below) The means of decades of acid output following histamine plotted against the means of the corresponding body-weights.

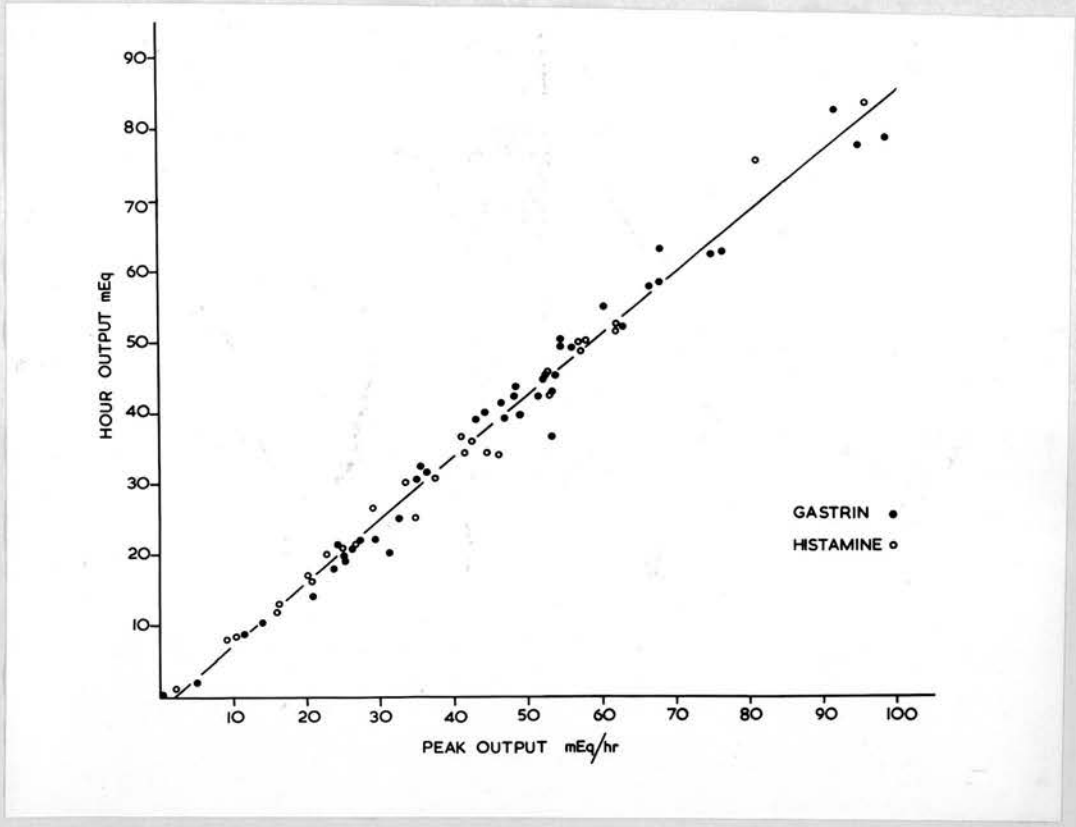
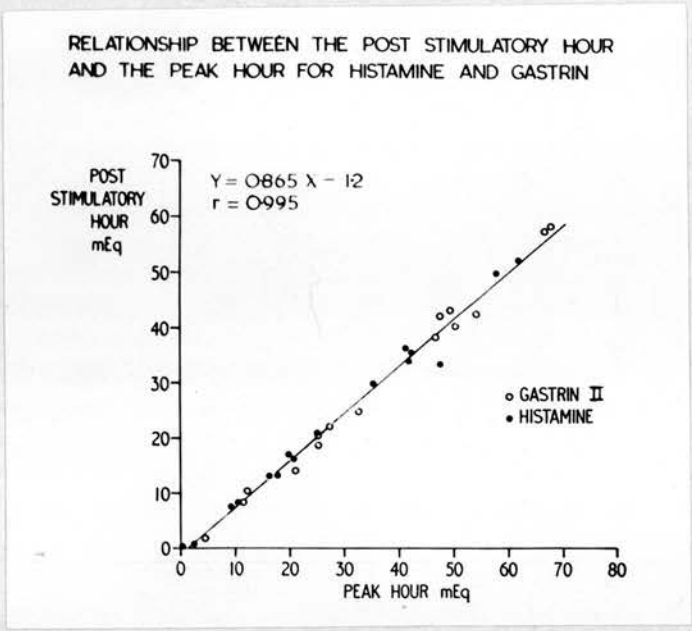


Figure 15 (a): The relationship between the post-stimulatory and peak hour outputs.
$Y = 0.877 X - 1.6$; $r = 0.991$; $n = 72$



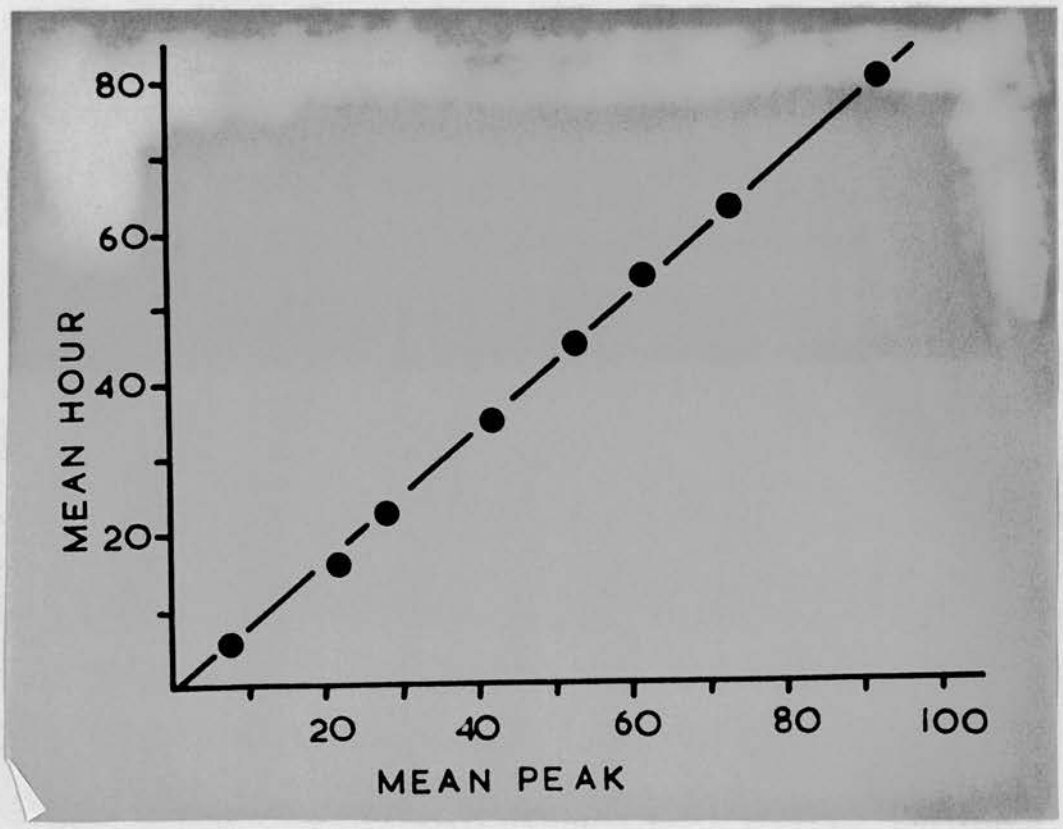


Figure 15 (b): (above) The relationship between the post-stimulatory and peak hour outputs for the series in Table A. The regression equation is nearly identical with that for all the data in Fig. 15a.

Figure 15 (c): (below) The means per decade of output of the data portrayed in Figure 15 (a).

CHAPTER VIII

THE EFFECT OF COMBINATIONS OF STIMULANTS ON GASTRIC ACID SECRETION IN MAN

In the comparative study of acid secretory responses in man, two important measurements against which all data are to be interpreted must, first be established with sufficient accuracy, namely, a base-line representing a reproducible and near-constant spontaneous secretion, and the maximal capacity of the stomach which is defined by the value of the calculated maximal response following stimulation by intravenous gastrin. These two measurements were obtained in subjects JM and GM (Ch. III to VI), and made possible the study of the effects of various combinations of stimulants.

The object of the study was to determine the extent of synergism between various stimulants of acid secretion and to confirm, further, the conclusion of the previous chapters regarding the parietal cell mass as the main determinant of maximal secretory response.

Methods

The problem was approached in two ways:

(i) A series of identical experiments, often repeated, were conducted on each subject, in which various combinations of gastrin, histamine and mechothane were administered subcutaneously. The dose levels used were usually those known to elicit a "maximal" response by this route, that is, 2 ug/kg for gastrin, 40 ug/kg for histamine/

histamine, 200 mg for histalog. These responses are subsequently referred to as the maximal subcutaneous responses. The following series of tests was performed:

- Gastrin, 2 ug/kg s.c.,
- Histamine, 40 ug/kg s.c.,
- Histalog, 200 mg s.c.,
- Gastrin, 2 ug/kg s.c. plus histamine 40 ug/kg s.c., administered simultaneously,
- Gastrin, 2 ug/kg s.c. plus mechothane 5 mg s.c.,
- Gastrin, 2 ug/kg s.c. plus mechothane by continuous infusion throughout the test in a dose of 3mg/hr
- Histamine, 40 ug/kg s.c, plus mechothane 5 mg s.c.

Subcutaneous mechothane was always administered 20 minutes prior to the test. The peak of parsympathetic activity as determined by 10-minute collections of saliva was clearly evident at the end of 20 minutes and coincided with the start of the secretory test.

Smaller dose levels of histamine and gastrin were also used and will be described in the appropriate sections.

Thirty-eight tests in all were performed, equally distributed between the two subjects. The tests were adequately randomised and ranged over a period of 20 months.

At the close of this series, the sera of both subjects were tested for the presence of antibodies to gastrin II by the method of Ganguli and Hunter [71], and found to be entirely free (personal communication)./

TABLE I. Comparison of the peak outputs following various modes of stimulation.

	Output/Hr GM	Output/Hr JM	% of Calculated Maximal Response			Ratio GM/JM
			GM	JM	Mean	
Calculated Maximal Response Infusion	36	54	100	100	100	66.6
" " " I.V. Gastrin	36	54	100	100	100	66.6
50-100 ug. I.V. Gastrin.	36	54	100	100	100	66.6
Maximal s.c. Gastrin.	32.9	49.1	91.4	91	91	67
Maximal s.c. Gastrin + maximal s.c. Histamine.	32.4	50.8	90	94	92	64
Maximal s.c. Gastrin + Mechothane s.c.	31.2	51	86.5	94.5	91	61
Maximal Histamine Infusion.	31.5	48.2	87.5	89	88.5	65.5
½-maximal Histamine Infusion + Mechothane.	31.7	46.6	88	86.5	87	68
Maximal s.c. Histamine + Mechothane s.c.	31.4	44.2	87	82	84	71
Maximal s.c. Histamine.	25.1	38.8	70	72	71	65
Histalog, 200 mg. s.c.	28.2	42.5	78.5	78.6	78.5	66.5
					Mean	66%

communication).

(ii) The synergistic effect of gastrin and mechothane was further investigated by constructing a dose-response curve for the combined effect of both stimulants. Four separate tests were performed on subject GM. Gastrin was given in single intravenous doses of 2,3,4 and 15 ug respectively on the same background of mechothane (5 mg subcutaneously, 20 minutes prior to the test).

A. The Secretory Response to Various Combinations of Stimulants

The peak responses from which comparison was made were calculated as indicated in Chapters IV to VI. The results are summarised in Table I and Fig. 1 and expressed for purposes of comparison as percentages of the calculated maximal responses of subject JM and GM respectively, which are thus taken to be 100.

(i) Intravenous administration of gastrin: It was indicated earlier that the calculated maximal responses following continuous infusion of gastrin were identical with those following single intravenous injections, and that they could be reproduced experimentally, by the intravenous injection of a single massive dose of gastrin.

(ii) Infusion of histamine or histamine plus mechothane: The maximal response to continuous infusion of 40 ug/kg/hr of histamine which appears in the last 20 to 30 minutes of the second hour, is only 88% of the calculated maximal response to gastrin. Its level, however, is identical with the level of the maximal response to continuous infusion of 20 ug/kg/hr of histamine/

histamine plus 3 mg/hr of mechothane, from which it can be inferred that the combination of histamine and mechothane is synergistic.

(iii) Subcutaneous gastrin plus histamine: The combination of gastrin and histamine is also synergistic at the lower dose levels. Thus, the response of subject GM to maximal subcutaneous histamine plus 0.25 ug/kg of gastrin was higher than the response to maximal subcutaneous histamine alone. When, however, the maximal subcutaneous dose of gastrin was administered simultaneously with the maximal or half the maximal dose of histamine, the response (92%) was not higher than that elicited by maximal subcutaneous gastrin alone (91%).

(iv) Subcutaneous gastrin plus mechothane: The administration of mechothane, subcutaneously prior to the test, or by continuous infusion throughout the test, appeared to have no effect on the maximal subcutaneous response to gastrin. When the effect was re-investigated using low intravenous doses of gastrin a distinct synergistic effect could be demonstrated (Fig. 4).

(v) Subcutaneous histamine plus mechothane: In contrast, prior administration of mechothane distinctly raised the subcutaneous maximal response to histamine to a level (84%) very near that achieved by continuous infusion of histamine (88%, Figs. 1 and 2).

(vi) Subcutaneous histalog: The maximal response to histalog (Fig. 2), is 78%, that is, in between the maximal subcutaneous gastrin and histamine responses. It bears the same relationship/

relationship to the maximal subcutaneous histamine response as has been previously demonstrated Ward et al. [72] .

Discussion

The demonstrable difference between the response of the intact human stomach to gastrin and histamine does not necessarily indicate an intrinsic difference in the response of the parietal cell to these stimulants. Similar differences have been encountered in the rat [61,73] . If it is accepted that gastrin acts by releasing histamine [74] , then, as Kahlson [61] points out, the difference may represent an intracellular mobilisation of histamine in proximity to the receptor sites, thus achieving a higher local concentration than is possible with histamine reaching the secretory cell from the outside. Inequalities in the rates of elimination and inactivation of the stimulants may also establish differences in their local tissue concentrations.

Since no dose-response curves were constructed for the effect of continuous infusion of histamine, it cannot be firmly concluded that the response obtained, or 88%, corresponds to the maximum theoretically possible following stimulation by histamine.

The subcutaneous responses to the highest doses and to various combinations of all stimulants were invariably lower than the intravenous responses. Subcutaneous administration may not achieve equivalent local tissue concentrations of the stimulant, and this may provide an explanation for the somewhat lower responses achieved under/

under this mode of stimulation.

The synergistic effects between gastrin and histamine and between histamine and mechothane were clearly evident and confirm similar findings in animals [58,75]. The synergistic effect between gastrin and mechothane, however, was more difficult to elicit at the high dose of gastrin used. At the level of the gastrin response (90 to 92%), a synergistic effect is probably minor and not easy to detect.

The low background tone, indicated by the absent or low spontaneous secretion in the two subjects, was probably responsible for the somewhat low subcutaneous histamine responses obtained and served to unmask the synergistic effect of mechothane on subcutaneous histamine. The responses of subjects with higher background vagal tone lie in between the subcutaneous histamine response and the response to subcutaneous histamine plus mechothane. The implication of these findings with respect to secretory tests is that the effects of fluctuations of background vagal tone are likely to be less pronounced on the higher subcutaneous gastrin response than on the subcutaneous histamine response.

Finally, the highly significant correlation between the post-stimulatory and peak hours established for gastrin or histamine administered singly, can also be shown to exist following administration of various combinations of gastrin, histamine and mechothane (Fig. 3).

B. <u>The Relationship between the Responses of the Two Subjects</u>

Maximal stimulation, implying, as it does in this context, activation/

activation of all available secretory units following a certain mode of administration of a stimulant, introduces a standard situation for comparing the responses of different individuals.

Inspection of Fig. 1 and Table I shows that a distinct relationship is present under all experimental conditions between the two subjects, namely, the fixed ratio of their responses under similar conditions of stimulation. It is suggested that this constant ratio, independent of the mode through which maximal stimulation is achieved, whether by single stimulants or combinations of stimulants, besides indicating the consistent pattern of response in the two subjects over a period of 20 months, represents, in effect, the sole discriminating parameter between them, namely, their respective parietal cell masses. An estimate of this on the basis of calculations provided by Shay [50], would give 1 and 1.5×10^9 cells for subjects GM and JM respectively.

C. Effect of Mechothane on the Response to Intravenous Gastrin

The subcutaneous administration of mechothane prior to a test raises distinctly the total and peak responses to an intravenous dose of gastrin (Fig. 4). Figure 5 shows the fit of the data obtained from four separate tests on subject GM to a logistic function. Figure 6 shows the linear relationship between the reciprocals of dose and response.

Discussion/

Discussion

(i) The maximal response calculated from the dose-response curve for the combined effect of gastrin and mechothane is identical with that calculated from the dose-response curve for the effect of gastrin alone. This is to be expected, if as the model predicts, maximal response is determined by activation of all available secretory units independently of how this is achieved. The parallelism between this finding and similar findings in animals is striking. Whereas simultaneous cholinergic stimulation potentiates the effect of small doses of secretin on canine pancreatic secretion, it has no effect on the maximal response elicited by injection of a large dose of secretin [76]. Similarly, eserine and other anti-cholinesterases [77,78,79] increase the salivary response to the lower doses of acetylcholine but are without effect on the maximal response elicited by administration of acetylcholine alone.

(ii) The slopes of the linear transformation of both curves in subject GM are also identical and indicate that, in either situation, the rate of the reaction between stimulant and secretory unit is monomolecular.

(iii) As expected, the location parameter for the combined effect is altered. The ED 50, or intravenous dose eliciting half the maximal response is 3 ug (0.047 ug/kg) for gastrin alone, and 2.4 ug (0.037 ug/kg) for gastrin plus mechothane. The difference indicates the extent of synergism induced by the subcutaneous dose of mechothane.

(iv)/

(iv) The linear relationship previously demonstrated between the reciprocals of dose and response is maintained. The single intercept on the vertical axis indicates that the reciprocals of the maximal responses are identical. The slope of the plot for gastrin plus mechothane illustrates graphically the extent of synergism.

(v) The term synergism is used here in the sense of co-operation, without any implications as to the mechanism by which such co-operation is mediated. It is not possible to predict to what extent the effect of mechothane is produced by antral release of gastrin, since little is known about alterations in antral pH during secretory tests. By analogy with animal experiments, however, it is probably true to say that the effect is, at least in part, produced by the influence of mechothane on the action of gastrin.

The mechanism by which the effect of cholinergic agents on the action of gastrin is produced remains obscure. The maximal response to histamine of a fully innervated canine pouch is not altered by subsequent denervation [35], but the dose required to reproduce it is much larger. On the other hand, vagotomy drastically reduces the maximal histamine response in man, while simultaneous cholinergic stimulation restitutes it to near pre-operative levels [80]. The effect of vagotomy on the maximal gastrin response is even more pronounced (over 90%) if the results of two tests on the same duodenal ulcer patient can be accepted as representative.

From/

From their extensive investigation of the combined effect of gastrin and urecholine or histamine and urecholine on the responses of denervated canine pouches, Gillespie and Grossman [58] concluded to the presence of true pharmacological potentiation. The assumption, implicit in the conclusion but difficult to substantiate, is that cholinergic agents adequately substitute for the exquisite physiological effect of the vagus.

Despite the advances in histological techniques, it is not yet known with certainty whether cholinergic nerve endings are present in sufficient proximity to the parietal cells for them to play a direct role in excitation. Neither is it known whether cholinergic agents are effective at the level of parietal receptor sites. The main evidence for the direct action of cholinergic agents is to be found in the experiments of Pevsner and Grossman [81] who injected acetylcholine directly into the gastric arterial circulation. In these experiments, however, the antrum was only isolated and not extirpated, and the possibility that some acetylcholine may have survived the general circulation and reached the antrum cannot be excluded. Further, the possibility that cholinergic stimulation by its effect on the intrinsic muscular tone of the stomach may release histamine locally by deformation of the amine-containing cells [82], or that it may facilitate access of the stimulant to intracellular sites, must be borne in mind.

While the shift in the location parameter shown by the dose-response curve in Fig. 5 can be explained on the basis of increased "reactivity" or "sensitivity" of the parietal cells to stimulation, there/

there is, so far, no experimental support for this assumption and little to recommend it besides conceptual ease.

On the other hand, several recent studies appear to support the view that the increased delivery of the stimulant consequent on alterations in mucosal blood flow, maybe the mechanism responsible for the synergistic effect exhibited by cholinergic agents. Holton and Jones [33] observed the effects of bradykinin, caffeine and vagal stimulation on the opacity of the stomach wall and on acid secretion in response to infusions of histamine or gastrin in dogs. Under suitable conditions, all three stimuli cause vasodilatation and increase the secretory response to gastrin and histamine. This finding confirms the earlier observations of Obrink [83] on the effect of priscol on histamine-stimulated secretion. Jacobson et al. [34] measured gastric mucosal blood flow by the aminopyrine method in dogs with denervated pouches. They observed a three-fold increase in mucosal blood flow following administration of urecholine with gastrin as compared with administration of gastrin alone. The effect of combinations of histamine and urecholine though distinct was less pronounced. These findings rejoin those of other workers [58,84] on the extent of synergism induced by simultaneous administration of urecholine with either gastrin or histamine.

If the view expressed above is correct, it implies that synergism is not the outcome of potentiation between two stimulants but simply the result of increased delivery of the same stimulant to the active sites of secretion.

NOTE ON INHIBITION OF GASTRIN-STIMULATED SECRETION.

A. The Effect of Anti-histamine on Gastrin-stimulated Secretion

The possible effect of anti-histamine on gastrin-stimulated secretion was studied on two sets of normal and ulcer subjects. Nine subjects were tested on two separate occasions with 2 ug/kg of gastrin. One test in each subject was preceded by an injection of 100 mg of mepyramine maleate. Ten subjects were tested with 2 ug/kg of gastrin and 40 ug/kg of histamine, preceded on both occasions by 100 mg of mepyramine maleate. The results, which are summarised in Fig. 7, were inconclusive, since only occasionally could a slight diminution of the gastrin response be demonstrated following administration of anti-histamine.

B. The Inhibitory Effect of Atropine on Gastrin-stimulated Secretion

Atropine has a marked inhibitory effect on gastrin-stimulated secretion. Subcutaneous injection of 0.01 mg/kg of atropine to subject GM prior to administration of the maximal subcutaneous dose of gastrin reduced the peak response by 60% (Figs. 1 and 8). A smaller dose of atropine (0.007 mg/kg) reduced the peak response of subject JM by 20%. These findings confirm an earlier report by Gregory and Tracy [36] that a dose of 1.2 mg of atropine given intramuscularly virtually abolishes the response to a submaximal dose of gastrin.

C./

C. Inhibition by Intravenous Gastrin

Inhibition of gastrin-stimulated secretion by a large intravenous dose of gastrin was tried on two separate occasions in the course of this study.

(i) The first attempt was conducted on subject JM at the close of a near-maximal infusion of gastrin. Over a period of 8 minutes, three successive intravenous doses of 40, 20 and 20 ug were injected rapidly. The subject collapsed without losing consciousness. The symptoms, which lasted around 15 minutes, included a sinking abdominal sensation, nausea and perspiration. Although the secretory response fell progressively over the next hour, it could not be attributed with any degree of certainty, in view of the presence of nausea, to the inhibitory effect of intravenous gastrin.

(ii) In a second attempt, the problem was approached with greater circumspection. Subject GM was given a subcutaneous dose of 0.25 ug/kg of gastrin, followed 40 minutes later by two intravenous doses of 50 ug each given over a period of 5 minutes and separated by an interval of 10 minutes. The response rose within 10 minutes to the highest level achieved in this subject and fell gradually over the next 70 minutes.

A patient with the Zollinger-Ellison syndrome, who had undergone vagotomy the year before, was given a near-maximal infusion of/

of gastrin (1 to 2 ug/kg/hr.) without showing certain evidence of diminution in her response.

It was conceived following these failures that the dose-inhibition curve might be represented by the mirror-image of the dose-response curve and that, consequently, the dose of gastrin required to elicit inhibition would be excessively high and probably unsafe to administer rapidly by the intravenous route in man. Confirmation of this prediction has since been obtained in rats by Morley et al. (personal communication).

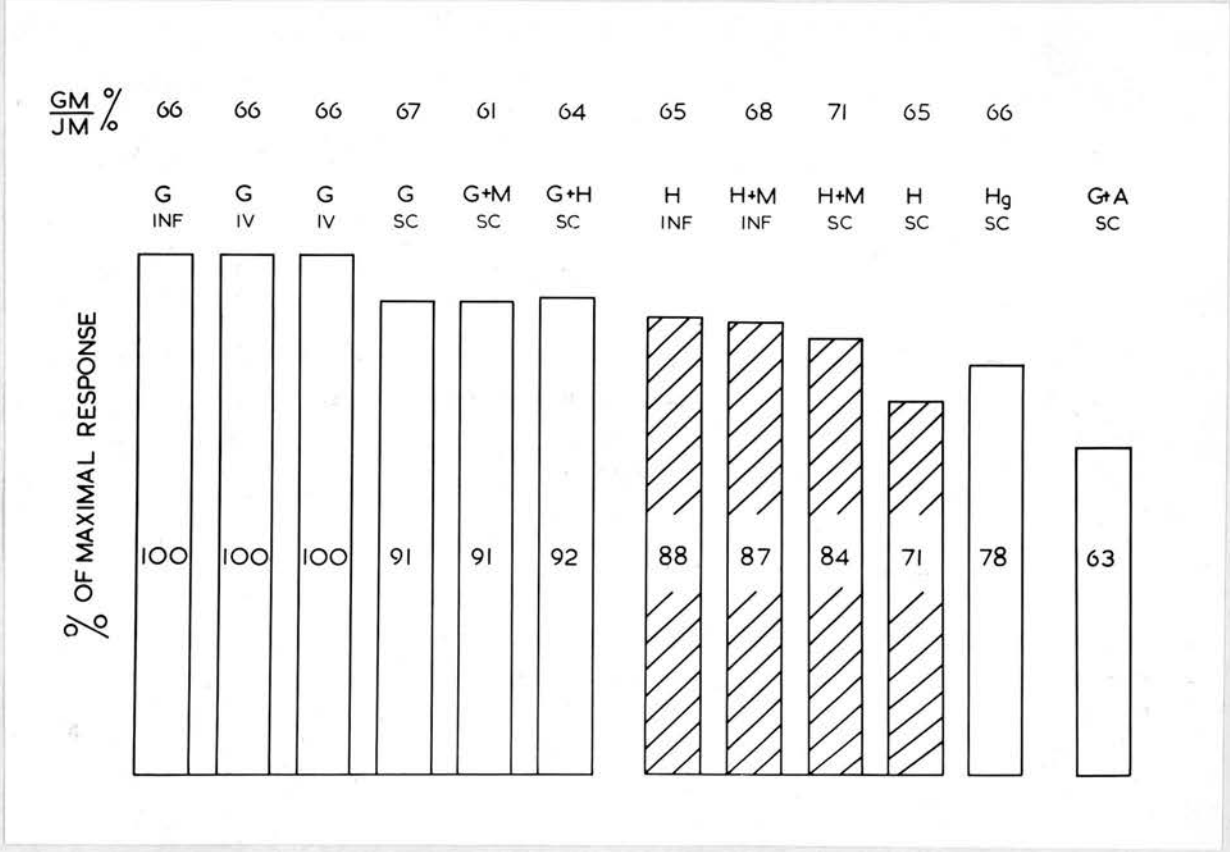
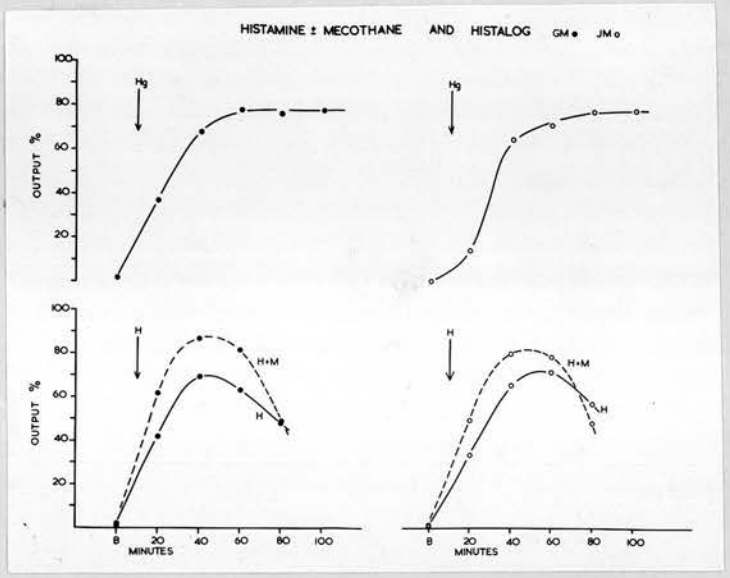


Figure 1: Histograms drawn from the data in Table I. The symbols G, H, Hg, M and A refer to gastrin, histamine, histalog, mecholhane and atropine respectively. The shaded part represents the data for histamine.



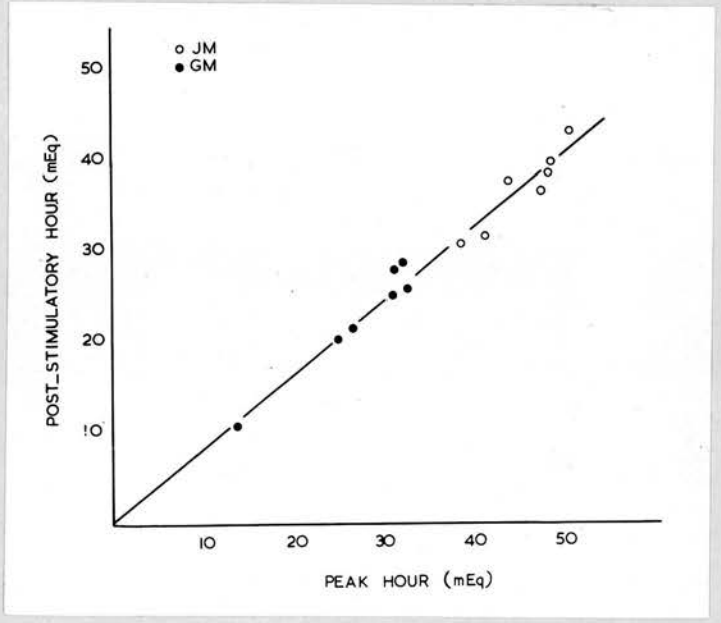


Figure 2: (above) The response to histalog (Hg) and the response to subcutaneous histamine and to subcutaneous histamine plus mechothane. Subject GM, closed circles; subject JM, open circles.

Figure 3: (below) The relationship between the post-stimulatory and peak hour outputs following various combinations of stimulants. r = 0.98.

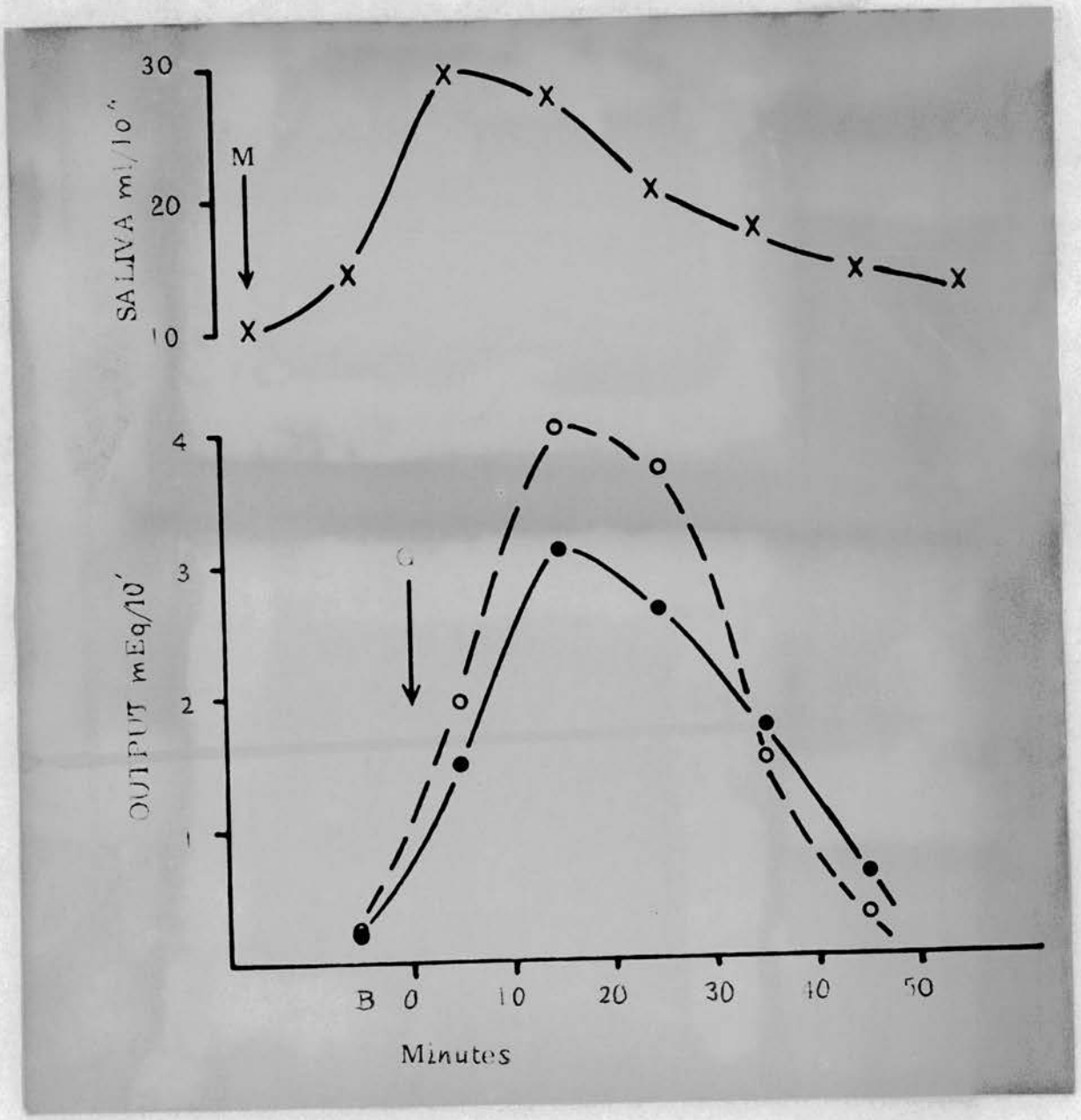
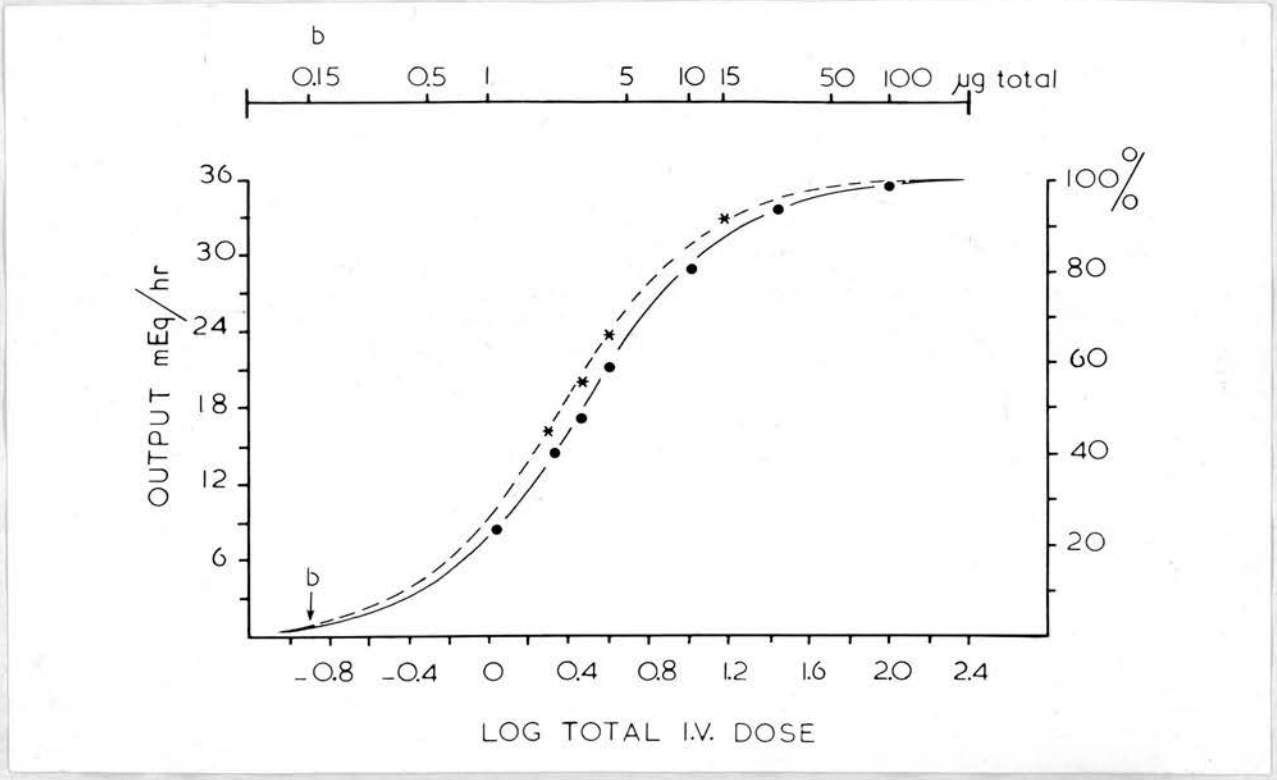


Figure 4: The synergistic effect of mechothane on the gastrin response. Closed circles, the response to 4 ug of gastrin intravenously; open circles, the response to the same dose of gastrin with prior administration of 5 mg of mechothane. The upper graph indicates the salivary response to the mechothane dose.



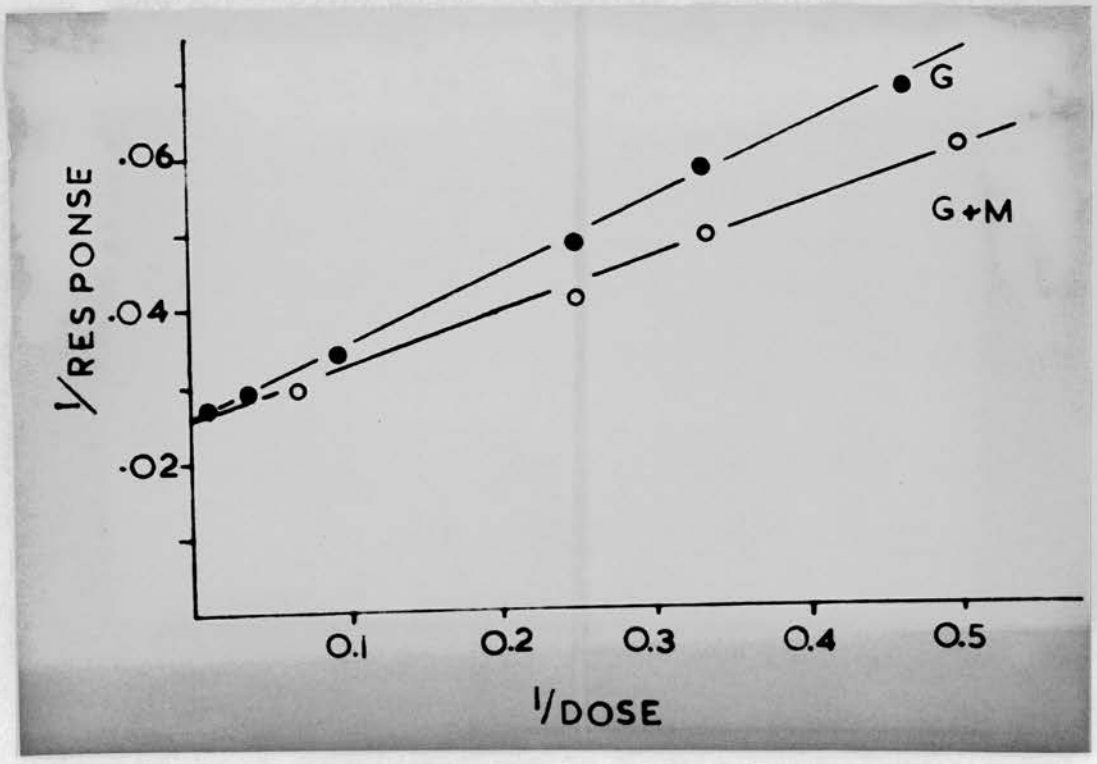
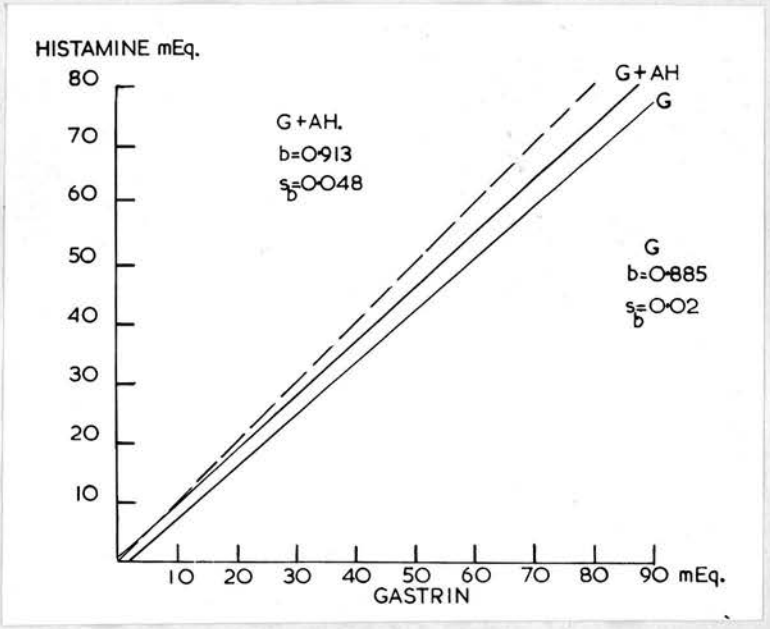


Figure 5: (above) Dose-response curves for the effect of gastrin alone (Closed circles) and gastrin plus mechothane (asterisks). The maximal calculated responses are identical

Figure 6: (below) The linear relationship between the reciprocals of dose and response, for gastrin (closed circles) and gastrin plus mechothane (open circles).



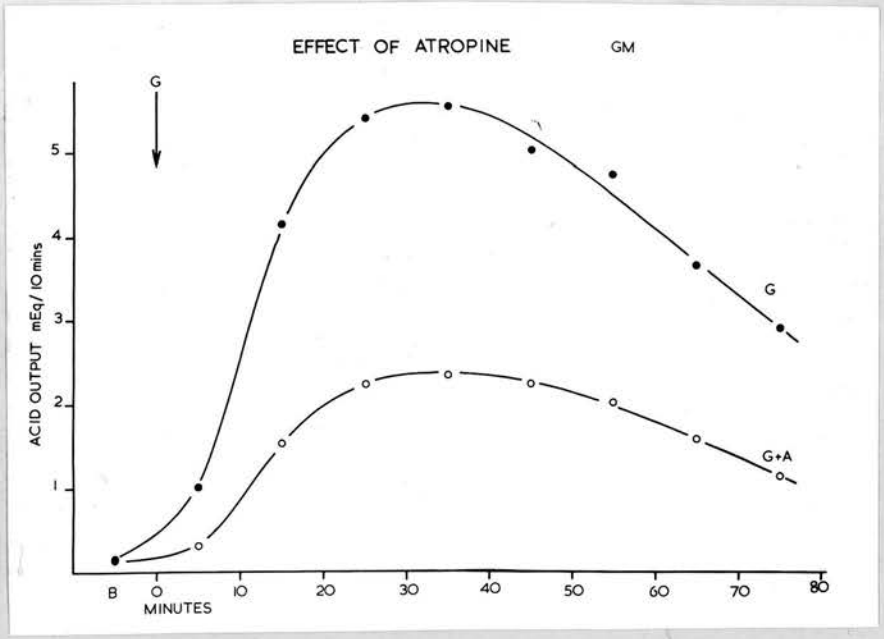


Figure 7: (above) The relationship between histamine output and the output of gastrin or gastrin plus anti-histamine.

Figure 8: (below) The inhibitory effect of atropine on the maximal subcutaneous response to gastrin in subject GM.

CHAPTER IX

THE SECRETION OF PEPSIN AND TOTAL NITROGEN FOLLOWING GASTRIN AND HISTAMINE

The correlative study reported in this chapter was undertaken (i) to determine the pattern of secretion of pepsin and total nitrogen following stimulation by gastrin or histamine, and (ii) to correlate the output of these products with the size of the cellular masses concerned.

The pattern of electrolyte secretion is described for purposes of comparison.

Methods

Six subjects (3 D.U., 2 G.U., 1 normal) were each tested on separate occasions with 40 ug/kg of histamine and 0.5 to 1 ug/kg of gastrin. The choice of the gastrin dose was dictated by the need to obtain a level of acid secretion equivalent to that following histamine.

The procedures for sampling of the juice and avoidance of salivary contamination were as outlined in previous chapters. Two 20-minute samples of basal secretion were collected prior to each test. Stimulated secretion was collected for 80 minutes, and 8 ten-minute samples were obtained for analysis in each case. The following estimations were made (see chapter on methods):

(i) H^+, Na^+, K^+ and Cl^- concentrations,

(ii) pepsin concentration,

(iii) total nitrogen concentration.

A./

A. Spontaneous Secretion

The mean pre-histamine basal output of acid was slightly higher than the pre-gastrin output. No significance could be attached to this observation as it represents the normal scatter in basal secretion. It is of interest, however, to note that the higher acid output was accompanied by almost exactly proportionate increases in the outputs of pepsin and nitrogen (Table I).

B. The Output and Concentration of Electrolytes, Pepsin and Total Nitrogen following Stimulation by Gastrin or Histamine

(i) Output:

The secretion of acid followed the pattern described in previous chapters. Following gastrin or histamine, the peak volume and acid outputs occurred simultaneously during the third and fourth 10-minute periods. The rates of rise and decline of secretion were, as usual, more rapid following histamine, but the mean total acid and volume outputs during the first hour were almost identical for the two stimulants (Table I).

The output of sodium following both gastrin and histamine rose sharply in the first 10-minute period to twice the basal level, but fell more gradually to a near-basal steady level during the latter half of the test. The mean post-stimulatory hour outputs of sodium for both stimulants were identical (Table I).

The patterns of secretion of potassium and chloride followed the pattern of acid secretion with only a slight tendency for the peak outputs of both ions to appear earlier following histamine.

The/

A. Basal Output/hr.	H^+ mEq	Na^+ mEq	Pepsin units	Nitrogen mgs.
Pre-histamine	5.05	5.7	15,134	49.7
Pre-gastrin	3.91	4.8	11,262	43.7

ooOoo

B. Stimulated Output/hr.	Vol. mls	H^+ mEq	Na^+ mEq	K^+ mEq	Cations mEq	Pepsin units	Nitrogen mgs.
Gastrin tests	369	44.9	7.0	6.4	58.3	35,340	105.0
Histamine tests	361	43.8	7.1	6.3	57.2	49,013	140.7
Ratio G / H%	102%	102%	99%	102%	102%	72%	75%

TABLE I.

A. Mean basal output per hour for the six subjects. The slight increase in the pre-histamine output of acid is accompanied by a proportionate increase in the outputs of the other constituents.

B. Mean outputs for the six subjects following administration of gastrin and histamine. The volume and electrolyte outputs are virtually identical. The post-gastrin, pepsin and nitrogen outputs are around 70 to 75 per cent of the post-histamine outputs.

The mean outputs of both ions were again almost identical for both stimulants (Table I).

The pattern of pepsin secretion was somewhat different from that of acid. Following both histamine and gastrin, the output of pepsin rose to an early peak in the second 10-minute period (Fig. 1). As for acid, the decline in pepsin secretion was more rapid following histamine. In all subjects, both the peak and post-stimulatory hour outputs of pepsin were noticeably lower (72%) after gastrin than after histamine (Table I).

The output pattern of nitrogen is similar to the general pattern of sodium and pepsin (Fig. 2). Again both the peak and post-stimulatory hour outputs of nitrogen are lower after gastrin than after histamine (Table I).

The pattern of output of all these constituents in individual subjects showed only minimal deviations from the general pattern described above.

(ii) Concentration:

The mean concentration levels per 10 minutes are listed in Table II. The lowest and highest concentrations achieved by all the constituents were virtually equal following both gastrin and histamine.

The hydrogen ion and sodium concentrations were reciprocal, with only a slight tendency for the hydrogen ion concentration to fall and the sodium concentration to rise during the latter half of a histamine test.

The potassium concentration peak preceded that of acid and its rate of decline was more rapid.

Following/

	10' periods	H mEq/L	Na mEq/L	K mEq/L	Cations mEq/L	Cl mEq/L	N mgs/L	Pepsin units/L
	Basal	44	79.0	9.4	132.4	132.2	725	162
Histamine	1	86	51.9	13.7	151.6	149.3	668	223
	2	120	18.0	20.1	158.1	158.0	460	184
	3	127	14.5	20.8	162.3	161.1	331	135
	4	127	15.0	18.9	160.9	161.2	307	112
	5	126	15.6	17.4	159.0	159.2	258	84
	6	122	20.0	15.4	157.4	156.8	311	83
	7	123	20.0	12.4	155.4	154.7	334	72
	8	120	20.8	10.3	151.1	151.7	390	75
	Basal	47	70.0	9.4	126.4	128.4	710	142
Gastrin	1	70	60.0	12.2	142.2	140.4	658	212
	2	109	26.5	17.9	153.4	153.0	400	132
	3	125	15.5	20.1	160.6	160.3	267	90
	4	127	15.5	19.0	161.5	160.6	261	92
	5	128	15.3	17.4	160.7	160.3	241	79
	6	129	15.7	16.2	160.9	160.3	238	75
	7	129	16.1	15.1	160.2	160.1	231	78
	8	129	15.9	14.5	159.4	159.7	261	85

TABLE II. Direct means of the concentrations per 10-minute period following gastrin and histamine.

Following both gastrin and histamine, the concentration of pepsin rose to a peak in the first 10-minute period, but the decline was more rapid with gastrin. The steady concentration levels achieved during the latter half of a test were similar for both stimulants and were roughly equal to half the basal level.

The concentration pattern of nitrogen was in all respects similar to that of sodium. The linear relationship between hydrogen ion concentration and the concentrations of sodium and total nitrogen is clearly evident in Fig. 3.

Discussion

The finding that, despite the near-equality in output of acid and other electrolytes, the output of pepsin was, in every subject, higher following histamine, must lead to the conclusion that at least this stimulant is an effective pepsigogue. This conclusion is supported by the data of Hirschowitz et al.[56] who reported a sustained output of pepsin during the entire duration of a histamine infusion. A similar conclusion was derived from the results of two maximal histamine infusion tests in subjects JM and GM. Infusion of gastrin, however, also led to a sustained, though lower, output of pepsin. It thus appears that both gastrin and histamine have, in different degrees, stimulatory effects on pepsin secretion.

If it is accepted that the peaks of acid output reflect the concentration level of the stimulant in the gastric tissues, then the early appearance of a peak of pepsin output and concentration indicates that part of the collected pepsin originated in a

preformed/

preformed product stored in the cells or deposited in the glandular crypts.

The nitrogen in the juice originates from the cellular (mucous and peptic cells) and extra-cellular (interstitial fluid) compartments of the stomach. The post-histamine output of nitrogen is more than the post-gastrin output, and reflects the higher content of pepsin and mucoprotein in the histamine-stimulated juice.

C. The Cellular and Extra-cellular Gastric Masses as the Determinants of Output

An important early observation by Card and Marks [62] was that the "maximal" acid output was linearly related to both the parietal cell mass and the mucosal volume of the stomach in man. An earlier study by Hunt [85] showed that the outputs of the parietal and non-parietal components were related to each other and to the output of pepsin. These findings pointed to the probability that the cellular and extra-cellular elements comprising the wall of the stomach were present in similar proportions in different individuals.

Maximal stimulation by gastrin or histamine introduces a standard situation for purposes of comparison. Under these conditions, the response of pepsin and other constituents of the juice may be assumed to be proportional to the size of the cellular or extra-cellular masses concerned. This prediction was put to the test on the data obtained in this study.

Basal output/

Basal Pepsin Output/hr. units	Test Pepsin Output/hr. units	Test Acid Output/hr. mEq.
2,200	20,000	19.0
6,900	23,100	24.3
11,200	40,000	39.5
13,900	41,700	45.3
15,800	48,000	58.3
29,200	81,500	80.5

TABLE III. The mean basal output of pepsin for each subject and the corresponding mean outputs of pepsin and acid obtained from the gastrin and histamine tests.

Basal Output:

In order to determine whether variations in the outputs of pepsin and nitrogen followed similar variations in the output of acid, the basal samples obtained (n = 24) were divided into three groups corresponding to their acid contents (0 to 3 mEq/hr, 3 to 6 mEq/hr and 6 mEq/hr and above). The linear relationship between the mean outputs of acid, pepsin and total nitrogen in the three groups is clearly apparent (Fig. 4).

If the pre-gastrin and pre-histamine samples are now grouped separately without reference to their acid contents the pepsin and nitrogen points obtained fall on the respective slopes in Fig. 3. Janowitz and Hollander [86] observed the same relationship between the basal outputs of acid and pepsin in a large series of normal and ulcer subjects, but found it difficult to interpret.

The dependence of the basal output of pepsin on the size of the peptic cell mass can be demonstrated by plotting the mean basal output of pepsin for each subject against the corresponding mean output of pepsin following stimulation by gastrin and histamine (Fig. 5, Table III).

Stimulated Output:

(i) Figure 6 shows the linear relationship between the output of pepsin and the corresponding output of acid in each subject during the first hour following stimulation by gastrin or histamine (Table III). The slopes of the lines denote the relative effectiveness/

effectiveness of gastrin and histamine as stimulants of pepsin secretion. The goodness of fit indicates that the peptic and parietal cell masses are present in similar proportions in different individuals and that there are no notable differences between individuals in the "reactivity" of their peptic cells to stimulation.

The data on pepsin output obtained following maximal infusion of histamine in subject GM and JM confirm these findings. The ratio of the output of pepsin in subject GM to that in subject JM was 66%, which, it will be recalled, is identical with the ratio of their maximal acid secretory responses. The results obtained by Crean [87] from direct peptic and parietal cell counts in rats amply confirm these predictions.

(ii) The output of sodium is similarily related to the acid output which indicates that the extra-cellular compartment, comprising the capillary network and the interstitial fluid, is related to the size of the stomach as expressed in the acid output (Fig. 7).

The output of nitrogen derived from the mucous cells and extra-cellular sources is also related to the acid output (Fig. 8). It should, however, be noted that unlike the pepsin values, the sodium and nitrogen values obtained from the two gastric ulcer patients fall on more acute slopes than those shown in Figs. 7 and 8, indicating that a greater leakage of interstitial fluid occurred in these patients.

The intrinsic factor was not estimated in this study, but it appears/

appears from the data of Wangel and Callender [88] that the output of this secretory product is also related to the acid output following gastrin and histamine.

Discussion

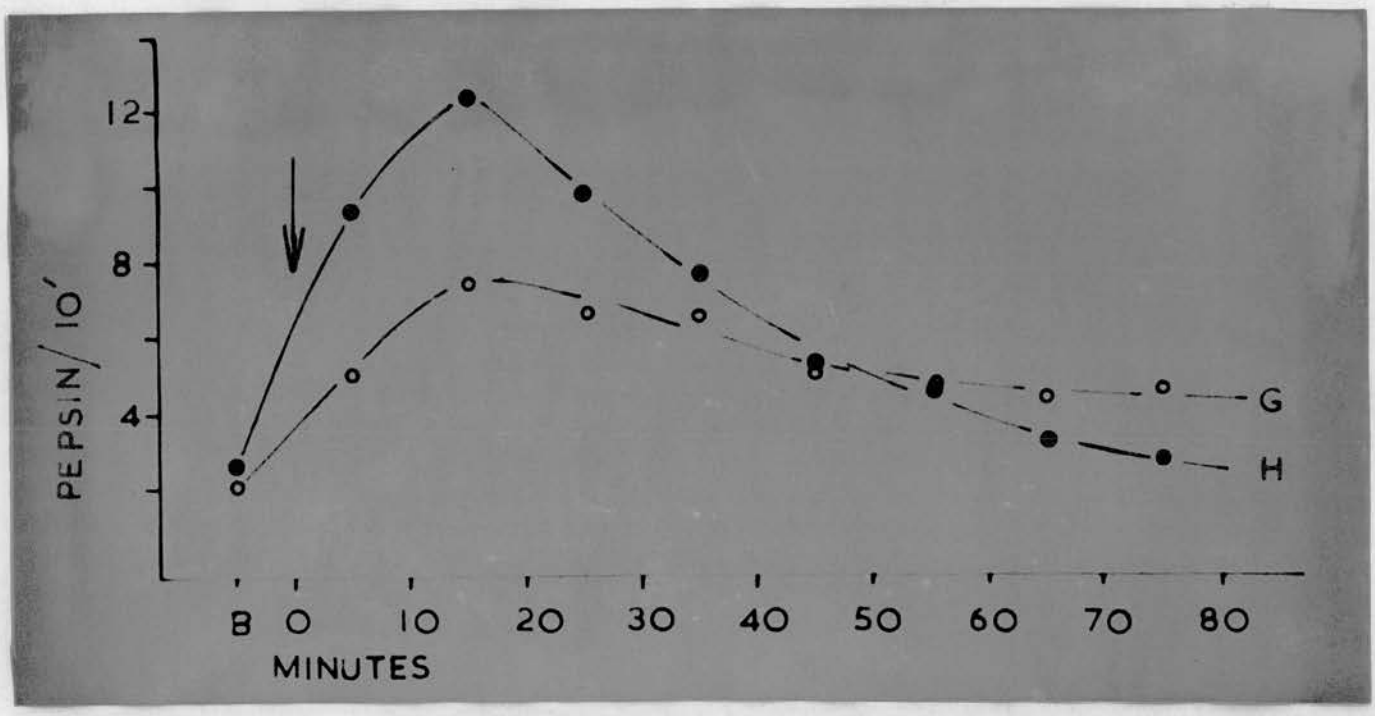
The conclusions from this study rejoin and confirm the conclusions of the previous chapters that the main, and under maximal stimulation, the sole determinant of response is the cellular or extracellular mass concerned.

The high acid output and the correspondingly high pepsin output in a duodenal ulcer population are related to the larger size of their parietal and peptic cell masses, which in turn, are related to the larger size of their body cell masses. It may, therefore, be suggested as a first approximation, that duodenal ulceration manifests itself in the context of a local and general problem of growth. Nothing is implied, however, in this statement as to the causal sequence of events. It is not known for certain whether duodenal ulceration precedes, or indeed is responsible, for the increase in the size of the stomach, although a number of observations and some recent experiments in animals appear to suggest it. Thus, acid output appears to be determined by the length of the history [89] and the proximity of the ulcer to the narrowest part of the stomach [70]. Experimental pyloric ligation in rats leads to a pronounced increase in the size of all the cellular masses comprising the stomach [87]. It is possible that the functional

or/

or organic stenosis that accompanies a duodenal ulcer may be a factor in determining the subsequent growth of the stomach, but it is difficult to visualise, in the state of present knowledge, how this factor could lead to a more general increase in body cell mass.

There is, however, little doubt that the cumulative evidence on this score must lead to a shift of emphasis in the study of the genesis of peptic ulceration.



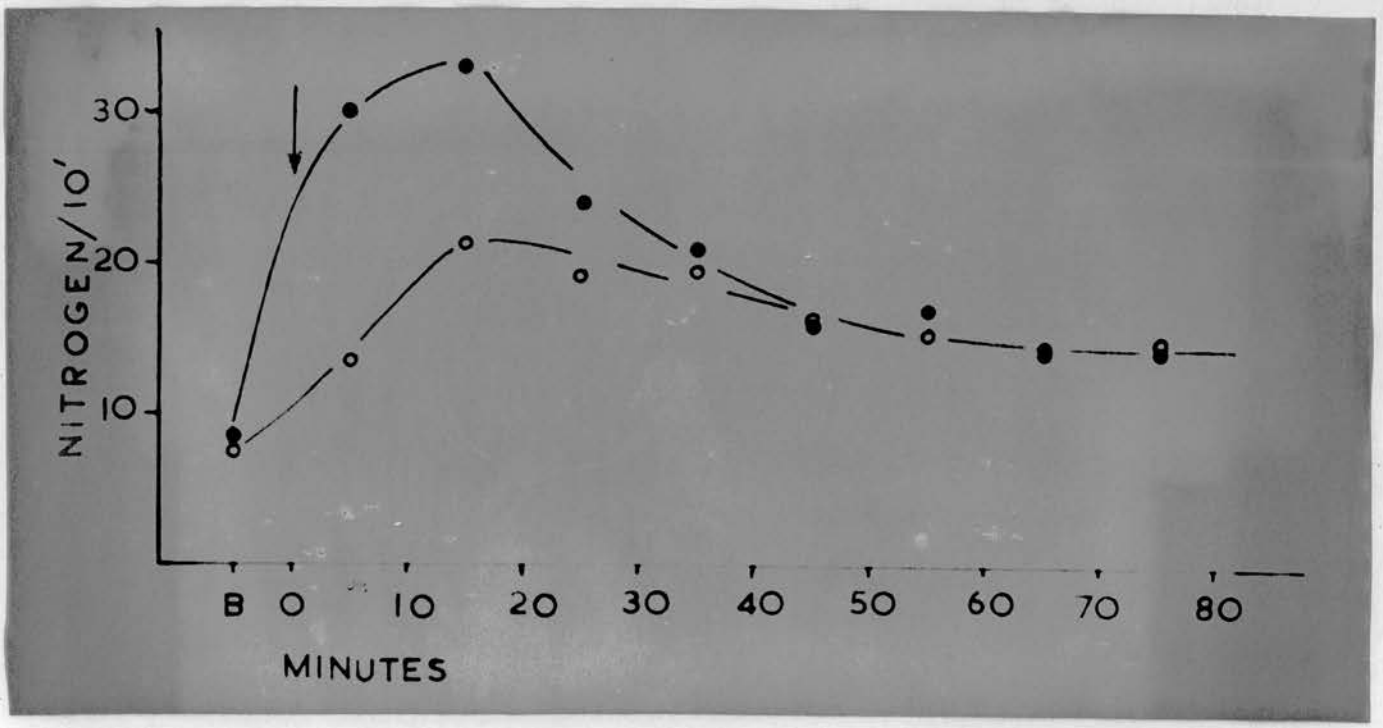


Figure 1: (above) Ten-minute output of pepsin following histamine (closed circles) and gastrin (open circles).

Figure 2: (below) Ten-minute output of total nitrogen following histamine (closed circles) and gastrin (open circles).

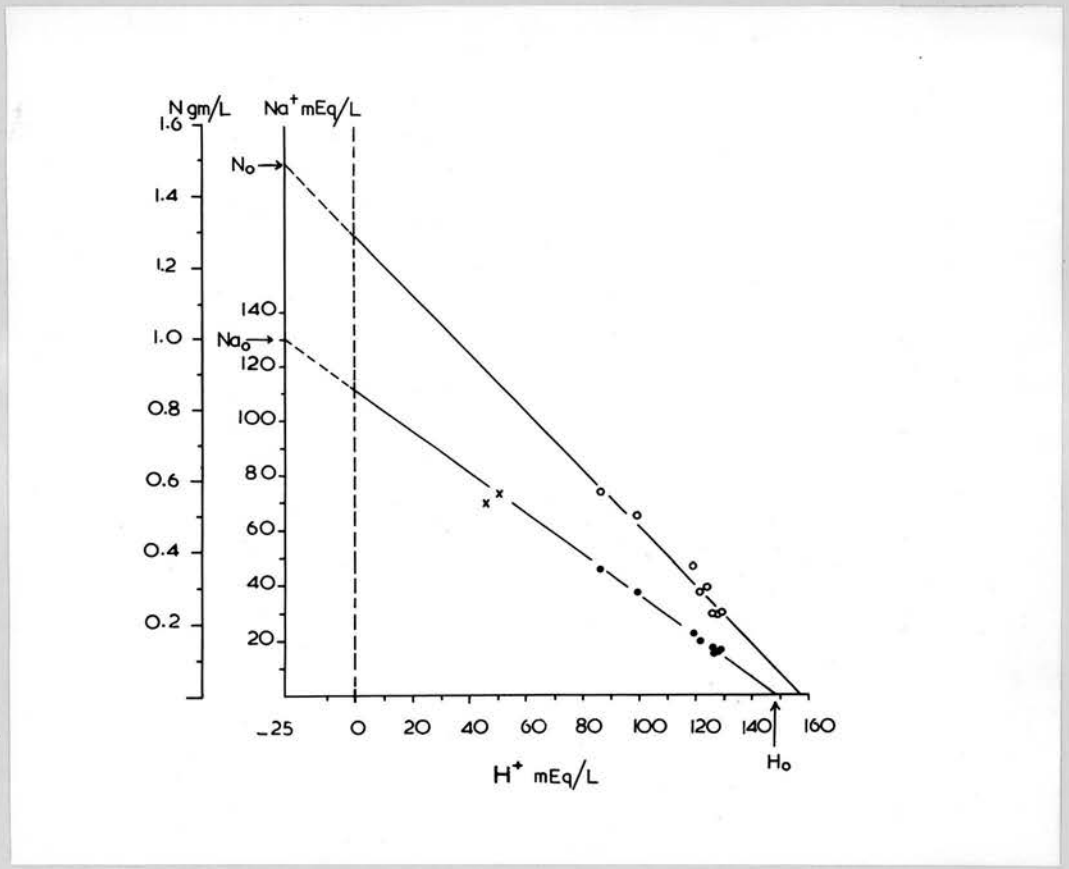
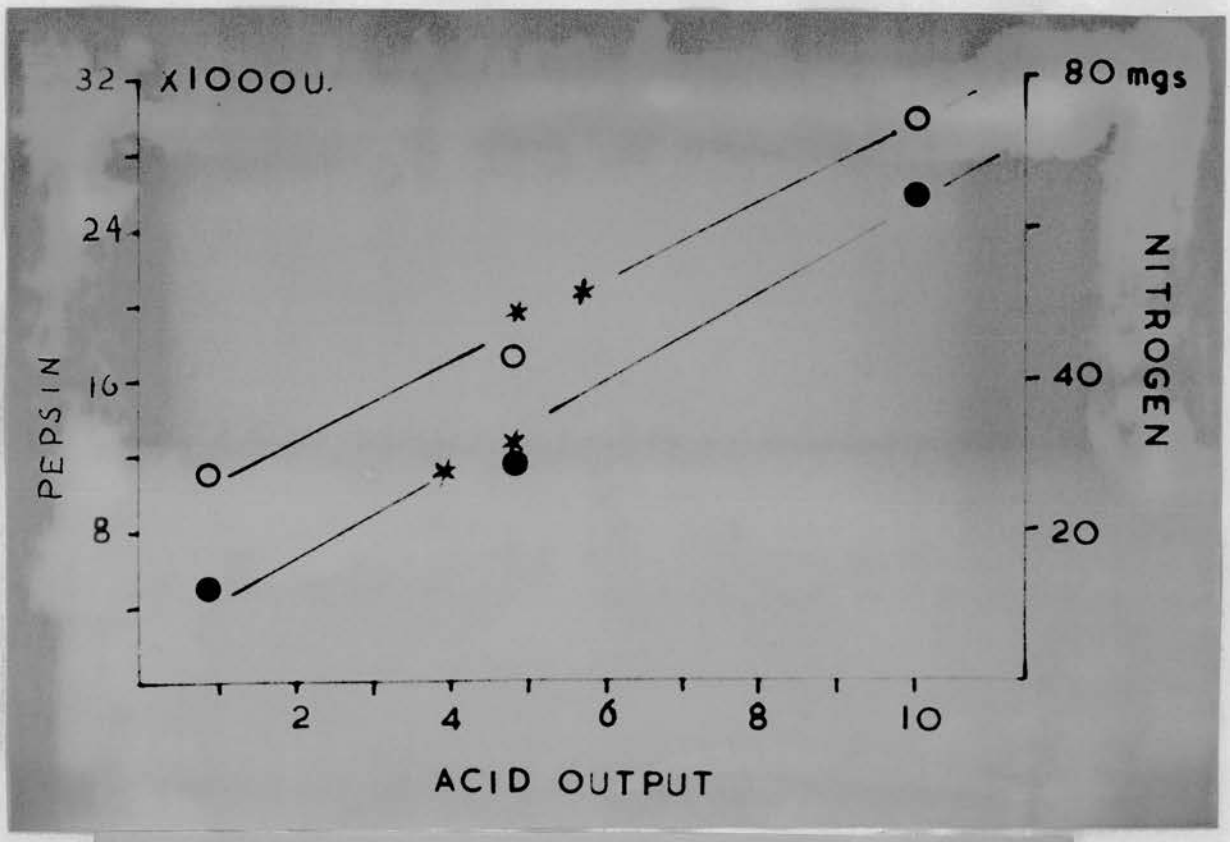


Figure 3: The linear relationship between hydrogen ion concentration and the concentrations of sodium and total nitrogen. The intercept on the vertical axis for sodium at -25 gives a value similar to the interstitial fluid value for this ion. The intercept for nitrogen on the vertical axis is similar to that obtained by de Graef [115] in dogs.



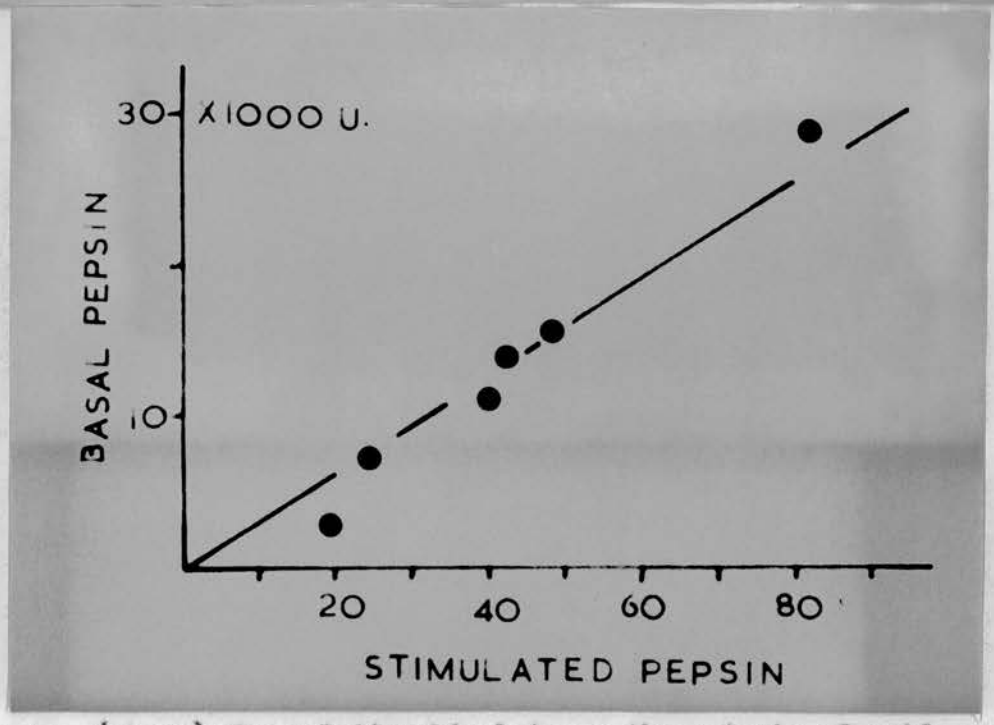


Figure 4: (above) The relationship between the outputs of acid, pepsin and nitrogen in basal secretion. The asterisked points represent the mean outputs in the pre-histamine and pre-gastrin tests.

Figure 5: (below) The relationship between the basal and stimulated pepsin output in the six subjects of this series.

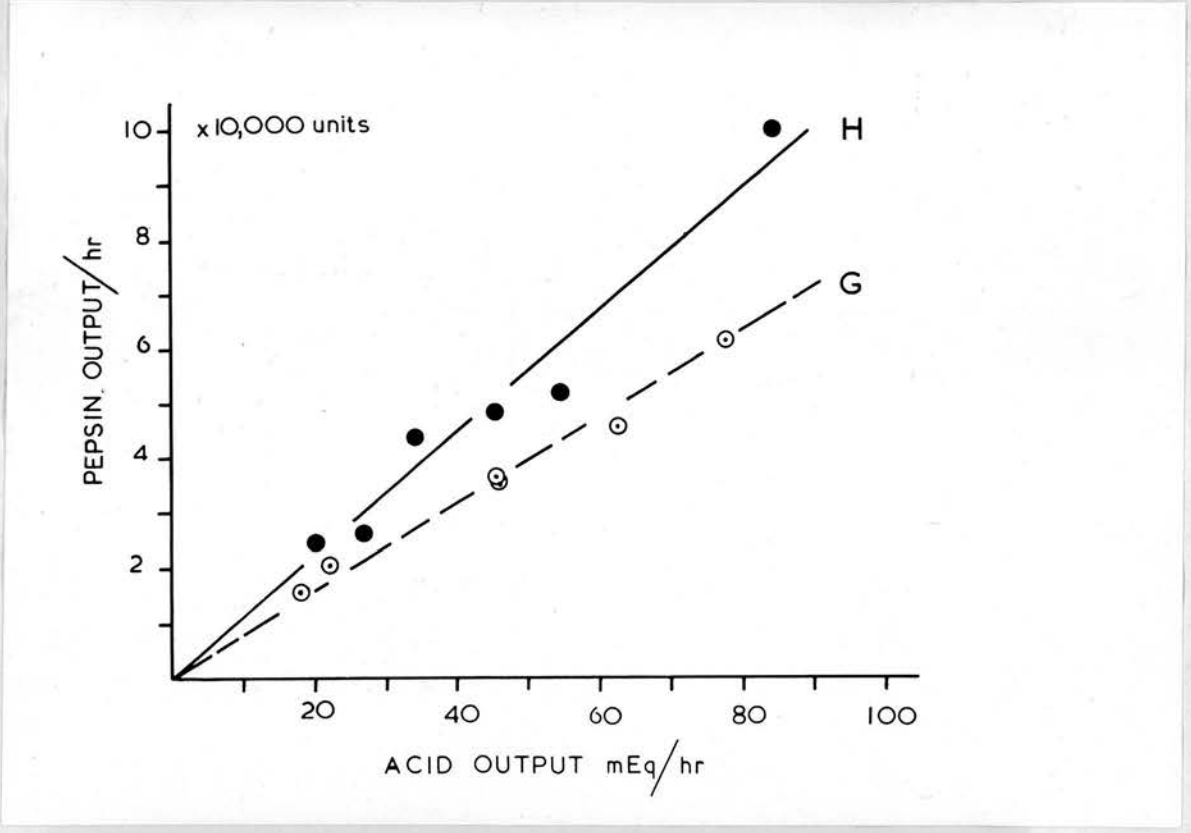
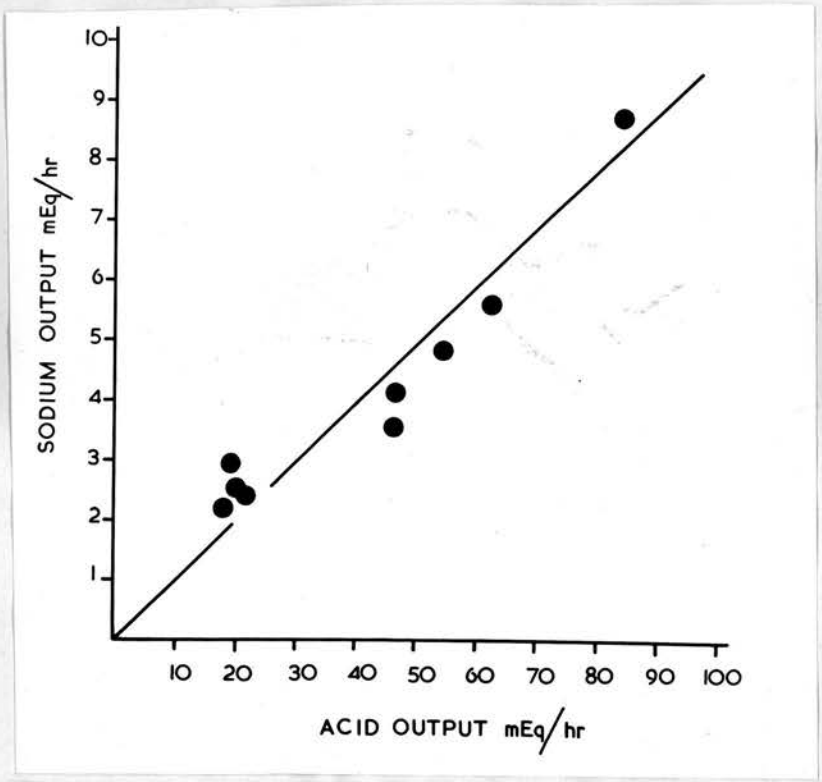


Figure 6: The relationship between the acid output following gastrin or histamine in each subject and the corresponding output of pepsin.



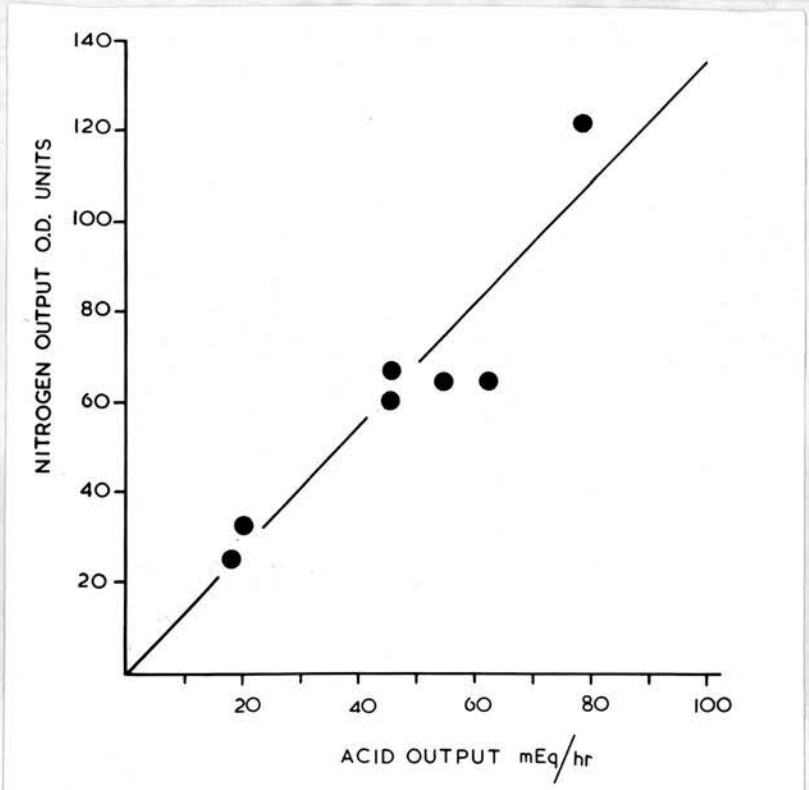


Figure 7: (above) The relationship between the output of acid and the corresponding output of sodium.

Figure 8: (below) The relationship between the output of acid and the corresponding output of nitrogen.

CHAPTER X

A QUANTITATIVE STATEMENT OF THE TWO-COMPONENT HYPOTHESIS OF GASTRIC SECRETION

The advent of gastrin offered an amusual opportunity for re-investigating the fundamental processes of gastric electrolyte secretion in man.

Section I

Gastric Secretory Rate and Electrolyte Concentration during Steady-State Secretion in Man.

Several authors have sought to characterise quantitatively the behaviour of gastric electrolytes in man and animals. Teorell [90] proposed a model, and a formulation for which he claimed only symbolical significance, in which a primary parietal secretion is altered by the back-diffusion of hydrogen ions and their replacement by sodium ions. An alternative hypothesis proposed by Hollander [91,92] maintains that variations in electrolyte composition are the outcome of a mixture of a parietal acid secretion and a non-parietal alkaline component. No adequate quantitative treatment has so far been given of this two-component hypothesis. A parietal statement of the quantitative implications of this hypothesis has been put forward by James [93]. Ihre's [94] data in man, on which Fisher and Hunt [95] attempted a quantitative analysis, were obtained during/

during non-steady-states, and lacked a full complement of ionic data and more especially sodium. Gray et al.[96,97] provided only a statistical analysis of electrolyte data obtained from denervated canine pouches.

An attempt is made here to provide a quantitative foundation for the two-component hypothesis. A unified treatment of the data will be given to account for the observed relationship between secretory rate and ionic concentration as well as between the concentrations of the various ionic species under all conditions of stimulation. Certain similarities between the two hypotheses will be shown and the differences discussed.

This approach is of more than theoretical interest since it underlies the interpretation of all secretory data obtained clinically or experimentally. Thus, according to the back-diffusion hypothesis, the parameter relating secretory activity and dose of stimulant is volume of secretion, while according to the two-component hypothesis, it is acid output; and depending on the circumstances these two parameters may vary independently.

The first section will be devoted to the study of gastric secretion under steady-state conditions, where a relationship between secretory rate and electrolyte concentration can best be demonstrated.

Methods

The two conditioned male subjects, GM and JM, were tested repeatedly/

repeatedly with a variety of stimulants over a period of 20 months. The tests involving infusion of gastrin II were all performed during the first 12 months. The range of doses of gastrin II delivered by continuous infusion was 0.2 to 90 ug/hr. The details of the procedures have been described in the previous chapters.

As previously described, the secretory response to continuous infusion of gastrin in man is biphasic, with a peak lasting 20 to 40 minutes, depending on the dose, followed by a more steady response. For this reason, only one dose level was used in any single experiment. In the early experiments, spontaneous secretion was collected for a period of one hour. As the subjects became conditioned to the procedure, it was found that a 20-minute collection, accurately recorded, provided a sufficient indication of the spontaneous secretion of acid and electrolytes. The following determinations were made: acidity by titration, using phenolphthalein as indicator; sodium and potassium by flame-photometer; and chloride electrometrically.

Results

A. The Pattern of Electrolyte Secretion following Infusion of Gastrin II.

The pattern of electrolyte secretion following continuous infusion of gastrin II is best expressed graphically. Except at the lowest dose levels, when a peak was slow to appear and difficult to detect, the pattern of secretion in both subjects was similar to that shown/

shown in Fig. 1. The values in Fig. 1 are calculated from the pooled data of the infusion experiments in subject GM. The secretory rate and outputs of acid and potassium show the biphasic response, with a peak followed by a near steady-state of secretion. The concentrations of acid and sodium are exactly reciprocal, as shown by the plateau achieved by their sum. The concentration of potassium, however, shows a peak coincident with the peaks of secretory rate and acid output. This is followed by a constant concentration value during the steady-state. This behaviour of the potassium results in a slight early peak of chloride concentration. The latter was in nearly every sample almost identical with the sum of cations H + K + Na.

B. The Pattern of Electrolyte Secretion following Infusion of Histamine.

It is of interest to compare the secretory pattern following a maximal infusion of histamine (40 ug/kg/hr) with that following infusion of gastrin (Fig. 2). Following infusion of histamine, the acid output in both subjects attained its highest level after a passage of nearly two hours. The concentrations of acid and sodium were reciprocal, and their sum rose to the highest steady-state value during the second hour. The potassium concentration showed an early peak similar to that following infusion of gastrin. The peak of potassium concentration preceded that of acidity and was soon followed by a steady-state value.

In/

In subject GM, the rise of secretory rate was rapid and the potassium output showed a peak response in the first hour similar to that following infusion of gastrin. In subject JM, whose spontaneous secretion prior to this test contained no acid, the rise of secretory rate was slower, and no early peak of potassium output, coincident with the observed peak of concentration, could be discerned.

C. Representative Data.

The following values were obtained during the highest steady-state secretions following infusion of gastrin II:

In subject GM.

65.5 mls/20 min: H , 131.8 mEq/L; Na , 12.5;
K , 17.1; Cl , 160.5

In subject JM.

101.5 mls/20 min: H , 141.7 mEq/L; Na , 8.0;
K , 14.9; Cl , 164.4

During the peak prior to the steady-state, the potassium concentration reached higher levels of up to 18 in subject GM, and 20 in subject JM. Similar levels of potassium were achieved following infusion of histamine, but the acidity was higher and the sodium concentration lower. The highest concentrations of sodium in this series were obtained at the lowest secretory rates during spontaneous secretion and coincided with the lowest acidity and potassium concentration/

concentration: 101 mEq/L of sodium for subject GM, and 120.5 for subject JM.

D. The Output of Sodium

The output of sodium, as the index of the non-parietal component, is of great importance in the analysis of the data and is reported separately. During spontaneous secretion, the output of sodium was not much different in the two subjects, and remarkably constant for each, even under conditions when no acid was present. In subject GM, the spontaneous output of sodium for the whole series and over a period of 20 months was 0.832 mEq/20 minutes ± S.E. 0.03, as compared with 0.816 mEq/20 minutes for the pre-infusion tests alone.

Following infusion of gastrin, the sodium output rises for a period of 20 minutes. This rise, which may reach up to three times the spontaneous level, is apparently dose-dependent (Fig. 3). During steady-state secretion, the sodium output falls again to a constant pre-infusion level despite the wide range of doses used, and a rise of secretory rate and acid output of up to sevenfold (Table I, Figs. 3 and 5). The mean sodium output of all samples obtained during steady secretion in subject GM was 0.813 mEq/20 minutes ± S.E. 0.02, a level indistinguishable from that obtained during spontaneous secretion. Spontaneous secretion in a conditioned subject is, in a sense, a low steady-state response to endogenous gastrin.

The steady-state output of sodium during maximal infusion of histamine/

Volume mls/20 mins.	H^+ Output mEq/20 mins.	Na^+ Output mEq/20 mins.
65.5	8.63	0.821
54.0	7.08	0.848
48.8	6.20	0.824
36.6	4.49	0.845
26.2	2.75	0.833
9.6 *	0.29 *	0.832 *

TABLE I. The mean steady-state output of sodium in mEq/20 minutes following infusion of gastrin II in subject G. M. The asterisked figure represents the mean output from 33 basal experiments in the same subject.

histamine, despite an early rise, was around 60% of that following gastrin.

Theoretical and quantitative analysis of the data.

The constancy of the sodium output under steady-state conditions leads to the conclusion that the non-parietal component is produced in fixed quantities. Under these conditions, the observed variations in electrolyte concentration are the resultant of an admixture of a variable dose-dependent parietal secretion with a non-parietal component of fixed output. A quantitative analysis based on this hypothesis follows.

The symbols H_o, K_p and Cl_p refer to the primary concentrations of acid, potassium and chloride in the parietal component.

The symbols Na_o, K_{np}, Cl_{np} and b refer to the primary concentrations of sodium, potassium, chloride and bicarbonate in the non-parietal component.

All these primary concentrations are constant.

k is the constant volume at which the non-parietal component is produced during steady-state secretion.

v is the observed total volume per specimen, i.e. the secretory rate, so that v - k is the parietal volume per specimen.

The brackets [ ] depict the observed ionic concentration in a specimen.

A. Acidity

If the observed variations in [H] are considered to proceed by a simple process of dilution, then the acid output could be described/

described by:

$$[H]v = H_o(v - k) \qquad \text{Eq. a.1}$$

$$= H_o v - H_o k \qquad \text{Eq. a.2}$$

$$[H] = H_o - 1/v \cdot k \cdot H_o \qquad \text{Eq. a.3}$$

$$= H_o(1 - k/v) \qquad \text{Eq. a.4}$$

From Eq. a.3 it can be deduced that the relationship of secretory rate, v and $[H]$ is hyperbolic, and that of the reciprocal of secretory rate or $1/v$ and $[H]$ is linear. $[H] = 0$ at $v = k$, when the total volume equals the volume of diluent, that is, in the absence of parietal secretion. Equation a.4 is identical with that derived by Obrink [60] on the basis of the back-diffusion hypothesis, where all the symbols retain their meaning, except for k, which is identified with the permeability coefficient.

B. If however the acidity changes are effected by a process of dilution and neutralisation, then the equations above could be extended to account for this dual process.

$$\text{Acid output} = [H]v = H_o(v - k) - kb \qquad \text{Eq. b.1}$$

$$= H_o v - k(H_o + b) \qquad \text{Eq. b.2}$$

$$[H] = H_o - 1/v \cdot k \cdot (H_o + b) \qquad \text{Eq. b.3}$$

From Eq. b.3 it can be deduced that the relationship of secretory rate v and $[H]$ is hyperbolic, while that of $1/v$ and $[H]$ is linear. The relationship of acid output and secretory rate v is linear (Eq./

(Eq. b.2). The value of k, the non-parietal component, is minutely different from the volume at neutrality (Fig. 4) and will approach it in proportion to the magnitude of the concentration of bicarbonate, b. The secretory rate will equal k at $[H] = -b$. If, as it appears likely, the bicarbonate concentration of the non-parietal component has a value not unlike its value in plasma or, more accurately, in interstitial fluid, that is, around 25 mEq/L for subject GM, then an estimate of k, the constant volume of the non-parietal component during steady-states, can be obtained.

C. Sodium.

In the intact human stomach, the observed sodium concentration appears to reach its highest values at the same time as acidity is approaching neutrality, that is, at some point on the X-axis (Fig. 4), some distance away from the origin. In subject GM, a sodium concentration of 101 mEq/L was recorded for an acidity of 18 mEq/L and a secretory rate of 8.5 mls/20 minutes. The highest sodium concentration for subject JM was 120.5, for an acidity of 13.2, and a secretory rate of 8 mls/20 minutes. It was noted above that the sodium output during steady-state secretion is constant:

$$\text{Sodium output} = [Na]v = Na_o k = \text{constant} \qquad \text{Eq. c.1}$$

$$[Na] = \frac{Na_o k}{v} \qquad \text{Eq. c.2}$$

from which it can be deduced that the sodium concentration varies through dilution by a progressively increasing parietal volume.

The/

The relationship of $[Na]$ and $1/v$ is linear, while that of $[Na]$ and v is hyperbolic. The sum of $[H]$ and $[Na]$ will tend to a value equal to H_o, the primary acidity, as the secretory rate attains its highest levels (Figs. 1 and 2). Thus, one sample obtained from subject JM, whose calculated primary acidity was 152 mEq/L, had an observed acidity of 150 mEq/L for a $[Na]$ of 2.8 mEq/L. The value of Na_o, the primary sodium concentration of the non-parietal component, may be obtained from the observed constant sodium output and the value of k as calculated from Eq. b.3. Alternatively, it may be obtained by the linear regression of $[Na]$ and $1/v$ (Eq. c.2), where at $v = k$, $[Na] = Na_o$.

D. Potassium

The output of potassium derives from both components:

$$\text{Potassium output} = [K]v = Kp(v-k) + Knp \cdot k \qquad \text{Eq. d.1}$$

$$[K] = Kp - 1/v \cdot k(Kp - Knp) \qquad \text{Eq. d.2}$$

From eq. d.2, it can be deduced that the relationship of $[K]$ and secretory rate v is hyperbolic, and that of $[K]$ and $1/v$ is linear. The relationship of potassium output, $[K]v$ and secretory rate v is linear (multiplying both sides of eq. d.2 by v).

At the highest secretory rates, $[K]$ tends to a value equal to K_p, its concentration in parietal secretion. At $v = k$, the $[K]$ equals K_{np}, the concentration of potassium in the non-parietal component.

E. Chloride and total cations

The chloride concentration at all levels up to neutrality is virtually/

virtually identical with the sum of the concentrations of cations (H + Na + K). For the present purpose, the minute levels of other anions and cations may be disregarded. The chloride, like total cations, has a dual origin from both components. It can be shown directly, or from the sum of equations b.3, c2, and d.2, that chloride concentration

$$[Cl] = H_o + K_p - 1/v \cdot k(H_o + K_p - Na_o - K_{np} + b) \qquad \text{Eq. e.1}$$

from which it can be deduced that the relationship of secretory rate and [Cl] is hyperbolic, and that of [Cl] and 1/v is linear. The relationship of chloride and secretory rate is linear. At infinitely high secretory rates, the primary parietal chloride Cl_p equals $H_o + K_p$, that is, the sum of primary parietal acid and potassium concentrations. At k = v, the concentration of Cl is at a minimum and Eq. e.1 reduces to

$$Cl_{np} + b = Na_o + K_{np} \qquad \text{Eq. e.2}$$

that is, the sum of the non-parietal anions, chloride and bicarbonate, is equal to the sum of non-parietal cations, sodium and potassium.

From Eq. e.1, it can be shown that osmotic pressure, coincident with the chloride concentration as the only anion, attains a maximum at the highest secretory rates and progressively falls until neutrality. Beyond this, it starts to rise again owing to progressive accumulation of bicarbonate.

F. Experimental data

It is clear from the good fit of the experimental data

(Table/

(Table II, Figs. 4 and 5) that all the theoretical predictions are fulfilled. The curves in Fig. 4, describing the relationship of secretory rate v and electrolyte concentration, are derived from the linear regression equations of 1/v and electrolyte concentration. The good correlation obtained justifies this treatment.

Fig. 5 shows the linear relationship derived above that exists between secretory rate and the outputs of Cl, H, K and K + Na. Under steady-state conditions, the output of sodium is constant and independent of secretory rate and is represented by a line parallel to the axis of secretory rate.

The primary concentrations in the parietal secretion H_o, K_p, Cl_p are represented by the intercept at $1/v = o$ of the linear regression equations relating concentration and 1/v (Table II). The value of k can be deduced from Eq. b.3 by assuming a concentration of bicarbonate in the non-parietal component analogous to that in plasma or interstitial fluid. The primary non-parietal concentrations are then derived by substitution for this value of k in the regression equations relating $[\overline{Na}]$, $[\overline{K}]$, $[\overline{Cl}]$ and 1/v. It is clear from the values thus derived that the composition of the non-parietal component approximates very closely to that of interstitial fluid. An identical estimate based on a much larger number of samples obtained from the same subject GM is given in the subsequent section. The full results are shown in Table II, section II.

The/

The calculated primary acidities following infusion of gastrin are 147.9 and 150 mEq/L for subjects GM and JM respectively (mean 149.0). The values of the intercepts on the volume axis (Fig. 4) are 6.7 and 7.9 mls/20 min. respectively (mean 7.3). These values are in close agreement with those obtained by Nordgren [98] following continuous infusion of histamine in five subjects: mean primary acidity, 150.2 mEq/L; intercept on the volume axis, 7.1 mls./20 min.

It was pointed out above that the value of the intercept on the volume axis (i.e. at neutrality) is slightly larger than the calculated volume of the non-parietal component k, and will approach it in proportion to the magnitude of b, the bicarbonate concentration. Assuming a value of 25 mEq/L for b, the output of this component is calculated to be 5.77 mls/20 mins. in subject GM and 6.77 mls/ 20 mins. in subject JM, the higher secretor of the two. At the highest levels of steady-state secretion, the parietal volume output is thus around 12 to 15 times the non-parietal volume output.

Discussion

(a) Whether the effect of the non-parietal component is one of simple dilution (Eqs. a.1 - a.4) or also of neutralisation by bicarbonate (Eqs. b1 - b.3) is immaterial to the fit of the equations, and is not immediately evident from the data. The calculated composition of the non-parietal component, however, will vary with the equation chosen. In the subsequent sections, evidence will be presented in favour of this component being alkaline and of comparable/

comparable composition to gastric interstitial fluid.

(b) It was noted above that an equation, identical with Eq. a.4, could be derived on the basis of the back-diffusion hypothesis [60], and is found to account well for the observed variations of acidity following infusion of histamine in man [98].

(c) Earlier, Teorell [90] had developed the following equation on the basis of the same hypothesis to account for the observed variations in sodium concentration in gastric juice:

$$[Na] = \frac{S_o}{v/r + 1} \qquad \text{Eq. f.1}$$

which reduces to $[Na]\,v = S_o r - [Na]\,r$ Eq. f.2

where S_o is the value to which the sodium concentration tends at secretory rest when $v = 0$, namely, the concentration of sodium in the interstitial fluid, and is thus identical with Na_o of Eq. c.1; r is the permeability coefficient of sodium in ml/min. and is smaller in magnitude than k, the permeability coefficient for hydrogen ions. At $v = r$, the sodium concentration $= S_o/2$, or around 70 mEq/L. Values, however, ranging from 100 to 120 mEq/L of Na were obtained in this study at secretory rates higher than k or r, and where the juice still possessed an appreciable concentration of H ions. Further, from Eq. f.2 it is predicted that the Na output, $[Na]\,v$, is linearly related to sodium concentration, and tends progressively to the highest value of $S_o r$. It thus appears on the basis of Eq. f.2 that the Na output is not constant but increases with increasing secretory/

secretory rates as the Na concentration tends to its lowest values. This is, however, contrary to the findings of the present experiments where the output of sodium during steady-state secretion was found to be constant.

The observed sodium concentrations find their best fit to Eq. c.2. A similar good fit to this equation can be demonstrated for the data obtained by Nordgren on human subjects (personal communication).

(d) The theoretical linear relationship derived above between secretory rate and the outputs of hydrogen, potassium plus sodium, and chloride ions, is confirmed by the present findings. No tendency of the line to curve towards the origin at the lowest secretory rates, as predicted by the back-diffusion hypothesis, can be shown (Fig. 5). It should be remembered in this connection that the main source of error in secretory experiments involves the under-collection of a sample at the lowest secretory rates. The accurate recording of the corresponding concentration tends to shift the point towards the origin. A similar source of error would shift the curve of secretory rate versus ionic concentration to the origin. In the data presented above, which span the entire course of the curve, no such shift to the origin is apparent (Figs. 4 and 5).

(e) It is of interest to compare the findings of this study in man with that of Gray et al. [97] on denervated canine pouches. In both studies, a linear relationship can be demonstrated (i) between the secretory rate and the output of acid, chloride and sodium/

sodium plus potassium; and (ii) between the concentration of hydrogen ions and the concentrations of chloride, sodium, and sodium plus potassium. Closer study of the 9 pooled specimens obtained by these authors reveals a virtual constancy of the sodium output despite a seven-fold rise of acid output. The slight variations found between specimens (0.36 mEq/20 min. ± S.D. 0.05) are probably due to the crude method of obtaining steady-state responses by repeated subcutaneous injections of histamine.

An estimate of the relative sizes of the canine pouches and the size of the intact human stomach in this study can be obtained on the basis of the respective maximal acid outputs following administration of histamine [64, 50].

It is probable that the output of the non-parietal component is also proportional to the size of the stomach [99]. The ratio of the output of the non-parietal component, i.e. the output of sodium, in these two studies is almost identical to the ratio of the maximal acid outputs. Thus, from the data of Gray et al. [97], for canine pouches of similar mean size to the intact human stomach of subject GM, the sodium output is calculated to be 0.86 mEq/20 mins which is closely similar to the steady-state output of sodium of 0.81 mEq/20 mins. found in this subject. This further argues in favour of a similar composition of the non-parietal component or interstitial fluid in the two species.

(f) The sodium output following infusion of single dose levels of histamine in cats [100] also shows minimal variability. Thus, for acid/

acid outputs of 5.3, 12.8, and 15.3 mEq, the sodium outputs were 1.41, 1.65, and 1.59 mEq. respectively.

(g) It will be recalled in this context that the net movement of sodium in experiments on chambered preparations of frog gastric mucosa is unaltered, despite changes in acid output and in the presence of secretory inhibitors [101].

Conclusions.

1. A quantitative statement of the two-component hypothesis is proposed.

2. The experimental data obtained during steady-state secretion following continuous infusion of gastrin in man show a good fit to equations derived on the basis of this hypothesis.

3. A preliminary estimate of the composition of the non-parietal component shows it to be similar to that of interstitial fluid.

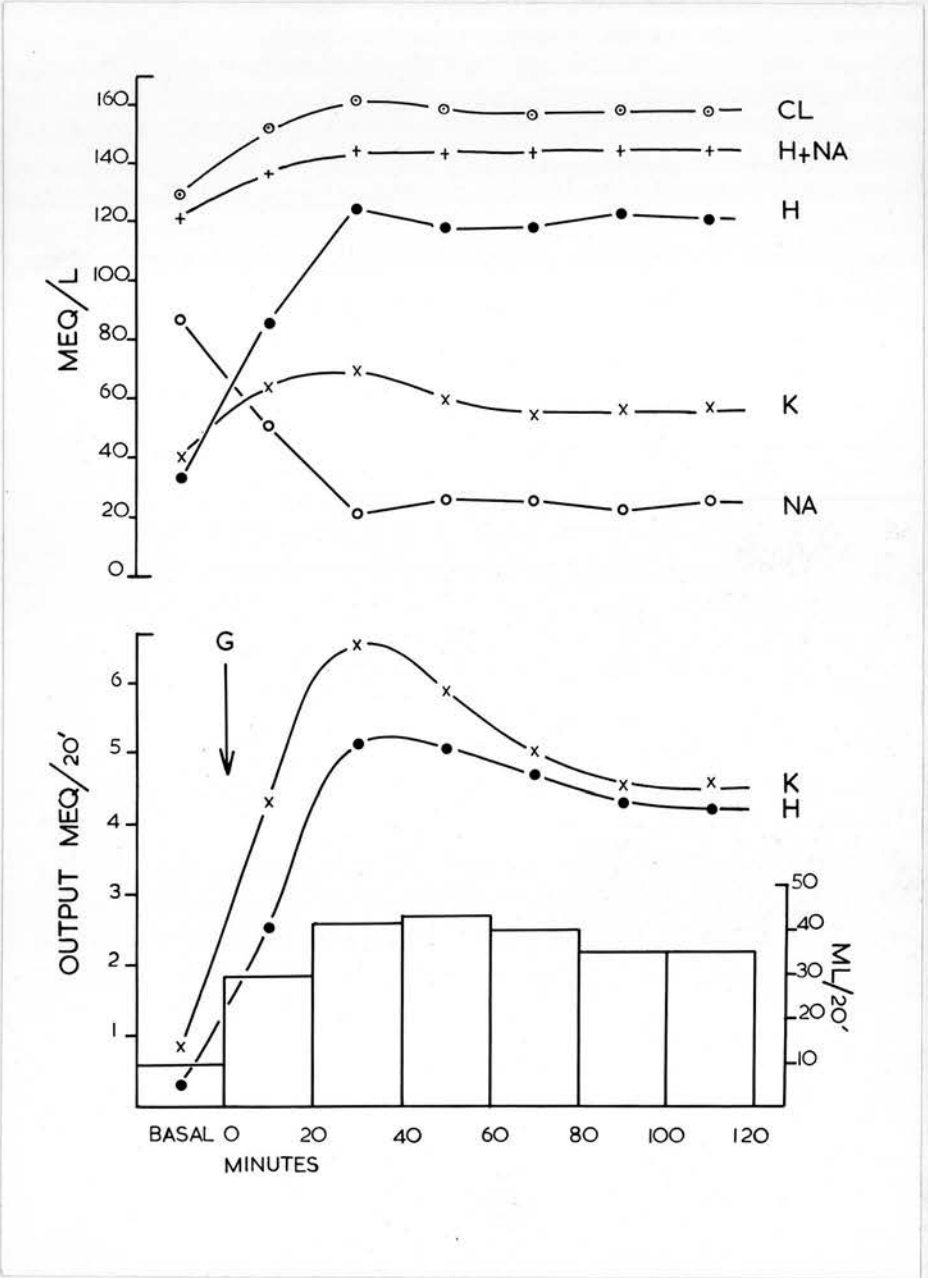


Figure 1: The pattern of secretion following infusion of gastrin II in subject GM. For purposes of illustration, the scale of potassium output has been increased tenfold and that of potassium concentration fourfold.

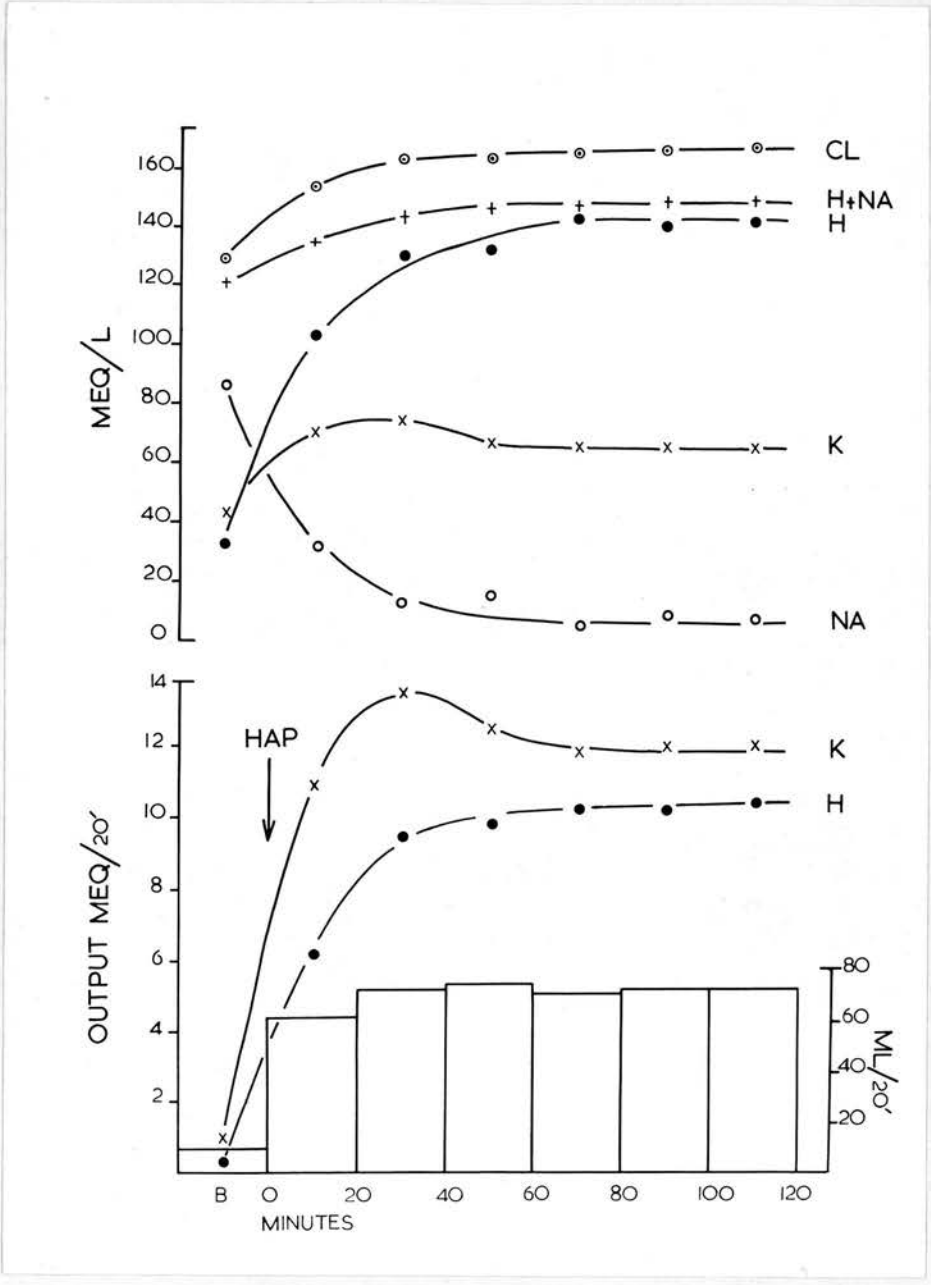


Figure 2: The pattern of secretion following infusion of histamine acid phosphate 40 ug/kg. in subject GM. The scale of potassium output has been increased tenfold and that of potassium concentration fourfold.

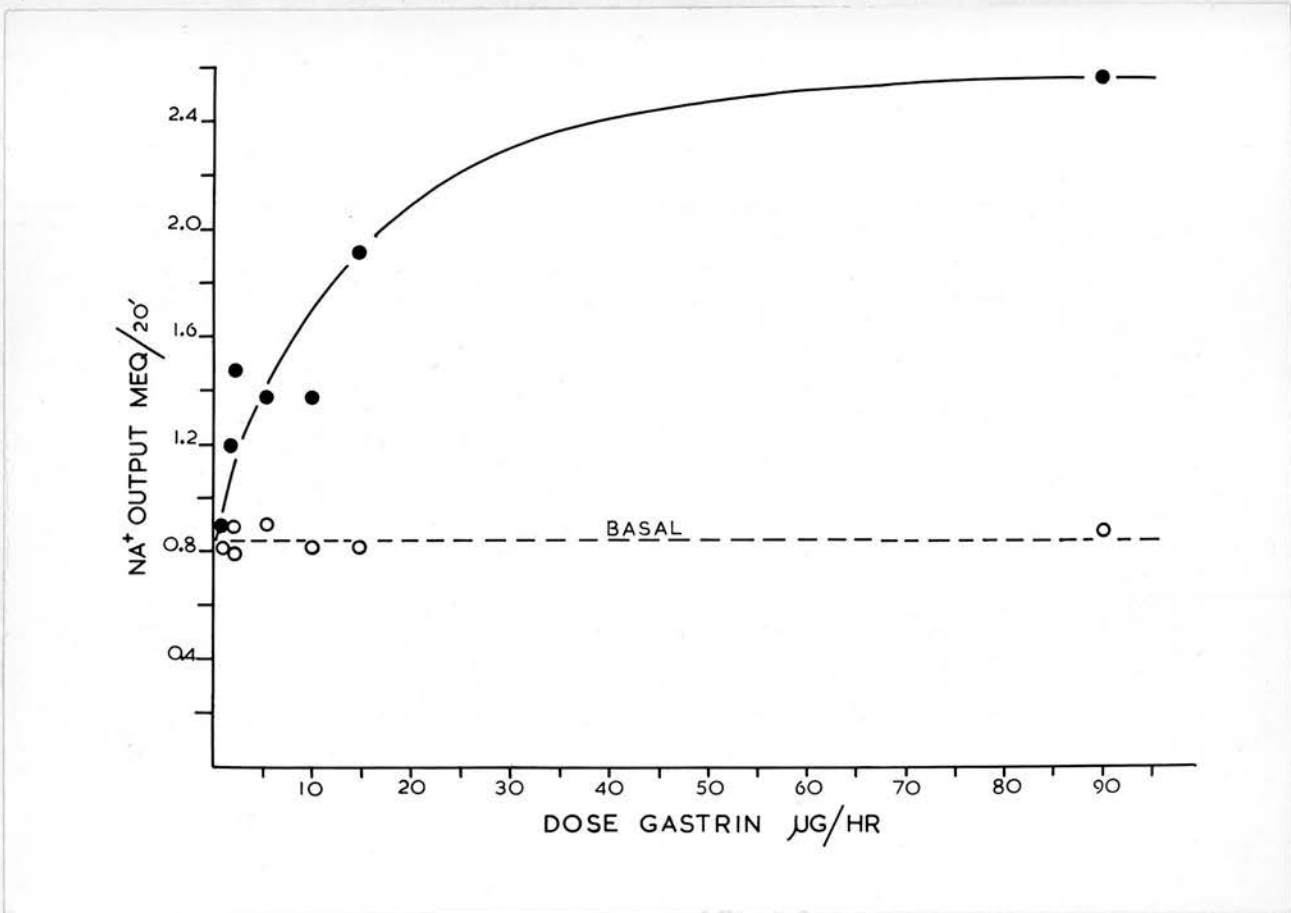


Figure 3: The relationship of sodium output to the infused dose of gastrin II in subject GM. The closed circles represent the output in the first 20 minutes. The open circles represent the mean output per 20 minutes during the remainder of the experiment. The dotted line represents the mean basal output per 20 minutes.

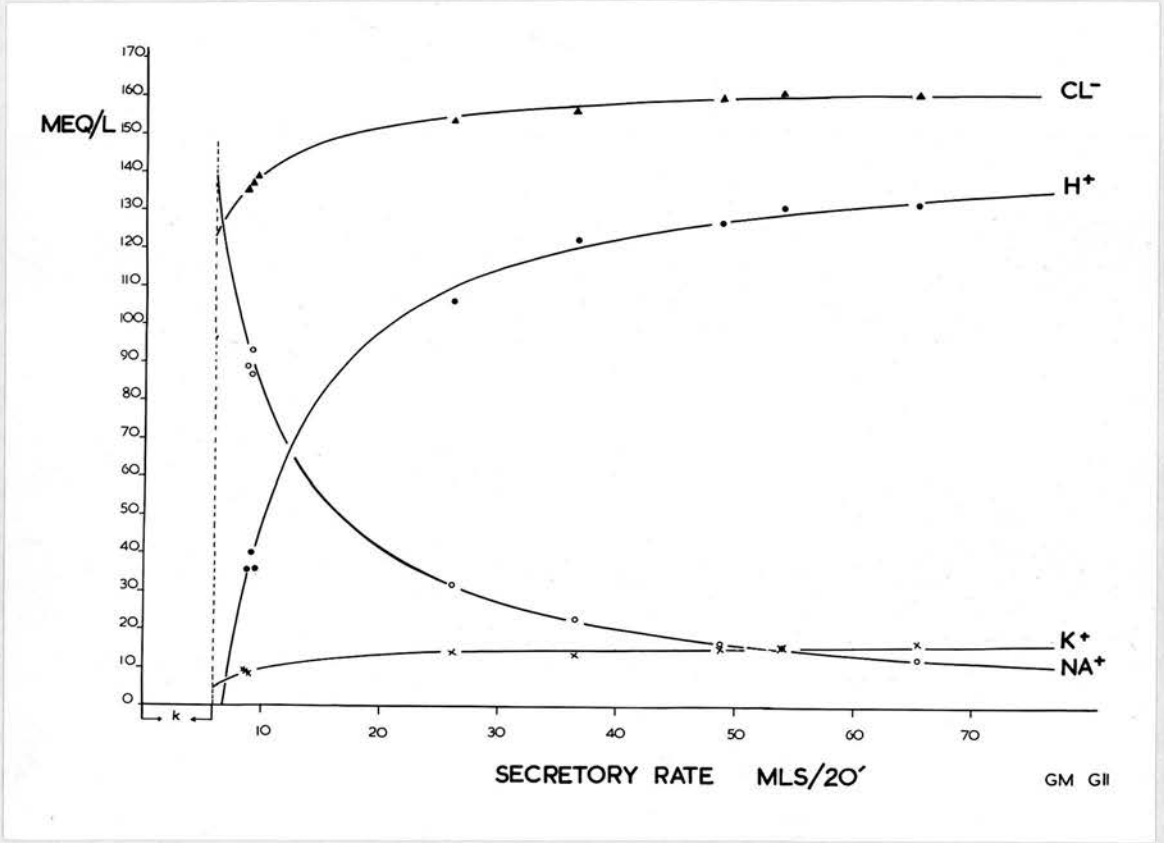
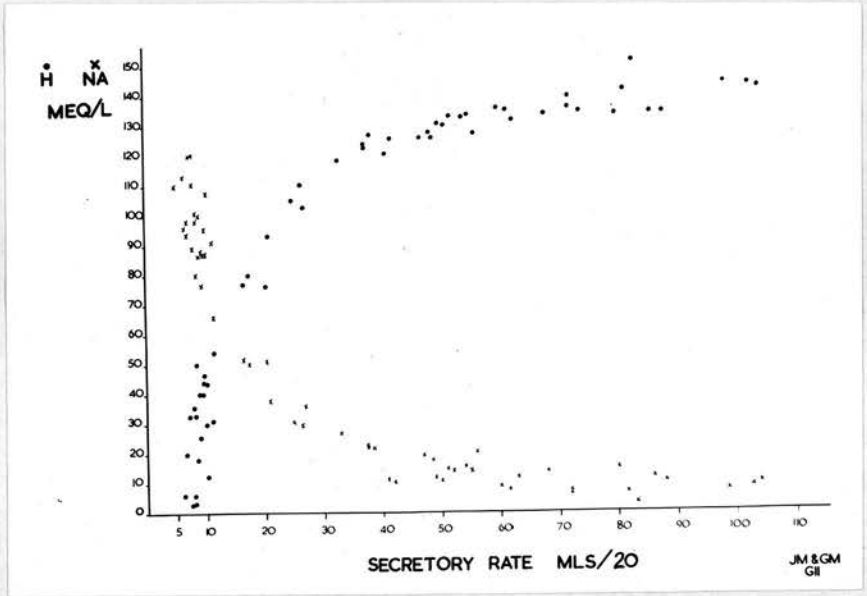


Figure 4: The relationship of electrolyte concentration to secretory rate following infusion of gastrin II in subject GM. The intercepts on the dotted line indicate the values of the primary non-parietal concentrations.



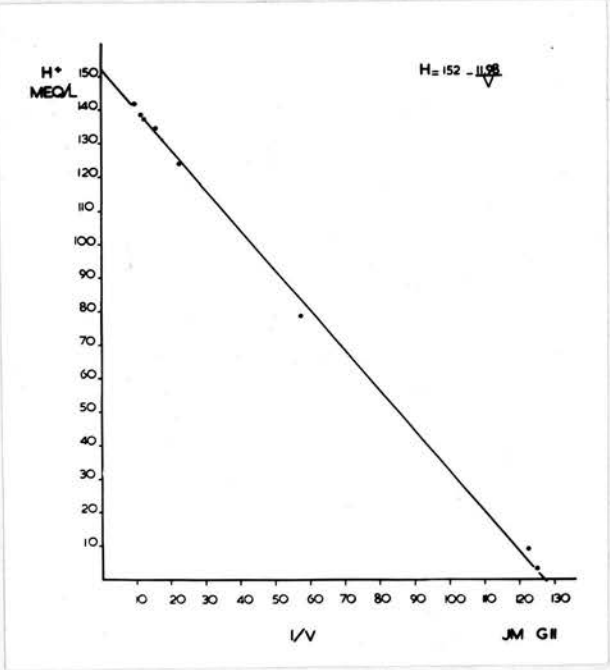


Figure 4 (b): (above) A scatter diagram of the hydrogen ion and sodium concentration data from subjects JM and GM.

Figure 4 (c): (below) The linear relationship between 1/v and the hydrogen ion concentration during steady-states of secretion (subject JM).

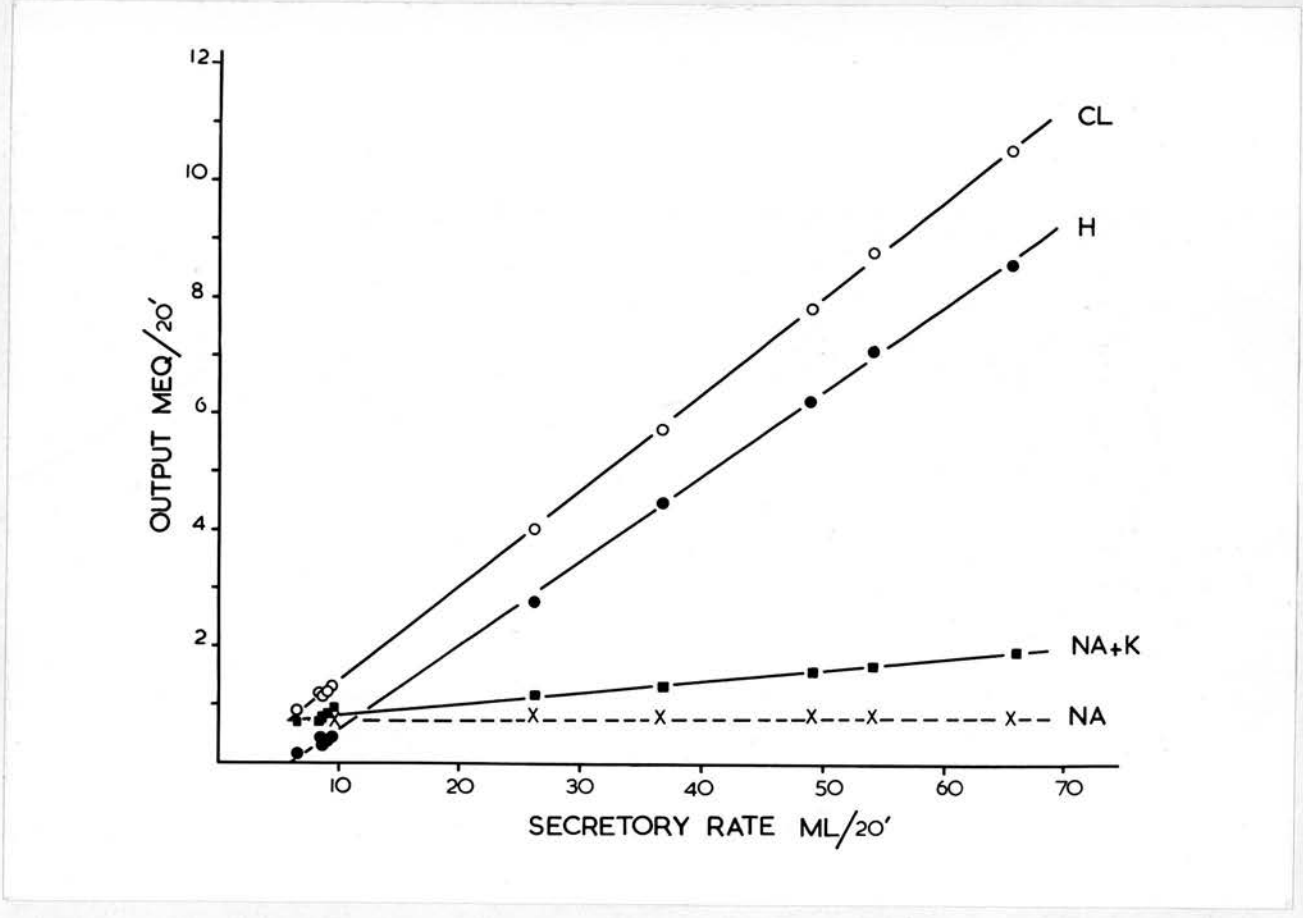


Figure 5: The linear relationship of secretory rate and electrolyte output in gastrin infusion experiments on subject GM. The constancy of the sodium output during steady-state secretion is evident.

Section II.

Gastric Secretory Rate and Electrolyte Concentration during Non-steady-state Secretion in Man.

In this section, the two-component hypothesis is further extended to account for the observed variations in electrolyte concentration and secretory rate during non-steady-state secretion induced by various modes of stimulation.

Methods

The procedures for collection of juice and avoidance of salivary contamination were the same as described previously. Biliary reflux was virtually absent in both conditioned subjects GM and JM, except in occasional samples following administration of mechothane.

Five-minute collections were taken throughout and pooled in samples corresponding to ten minutes for estimation of electrolyte concentration, except in the case of single intravenous injections of gastrin when five-minute samples were estimated directly. Spontaneous secretion was collected for 20 minutes at least prior to every test.

In both subjects, gastrin II was given by subcutaneous or single intravenous injection, either alone or in combination with histamine or mechothane (carbaminoyl E-methyl choline chloride). Histamine was given by subcutaneous injection alone or in combination with mechothane. The subjects were also tested with insulin

(0.1/

(0.1 unit/kg. i.v.) and histalog (200 mg. s.c.).

For purposes of comparison with the results in section I, and in order to avoid intersubject variability, the data obtained from subject GM will be presented mostly. The data from subject JM were substantially similar.

Results

A. Stimulation by Single Intravenous Injections of Gastrin

This mode of stimulation results in the opposite extreme of a secretory steady-state. The pattern of secretion is best illustrated graphically (Fig. 6). The secretory rate rises rapidly to a peak in the second five-minute period. The peaks of acid and potassium outputs occur simultaneously during the fourth five-minute period. This apparent discrepancy between secretory rate and acid output is due to the relatively large proportion of non-parietal component in the first few samples. Thus the output of sodium in the first 10 to 20 minutes rises to nearly three times "basal" levels, only to fall again to very nearly that level for the remainder of the secretory period.

The potassium concentration rises rapidly to a peak which just precedes the peak of hydrogen ion concentration. The concentration of sodium is exactly reciprocal to that of acid. Towards the end of the secretory period, both $[H]$ and $[K]$ fall to very low levels ($[H]$ = 15-20 mEq/L; $[K]$ = 5-6 mEq/L), while $[Na]$ rises to some of the highest levels achieved in this subject (106 - 108 mEq/L).

This secretory pattern is reproduced over nearly the entire range of dose levels.

B/

B. Stimulation by Histamine, Histalog, Insulin, and Subcutaneous Gastrin.

The secretory pattern following stimulation by subcutaneous histamine or histalog is similar to that reported by a number of workers [102] (see previous chapter).

The pattern of volume and acid output following stimulation by subcutaneous gastrin was reported earlier. Although gastrin-stimulated juice can be highly acid, the highest acidities and lowest concentrations of sodium were usually obtained following administration of large doses of histamine or histalog ($[H]$ = 142-147 mEq/L; $[Na]$ = 3-5 mEq/L). The simultaneous administration of mechothane, either subcutaneously or by continuous infusion with either histamine or gastrin, invariably lowers $[H]$ and raises $[Na]$.

B. Output of Sodium.

(i) The output of sodium during the first hour of a secretory period appears to vary with the nature of stimulant or combination of stimulants used. Table IV lists the data from a series of experiments in subject GM. The output of sodium following subcutaneous administration of 2 ug/kg of gastrin is higher than that following histamine. Mechothane especially, when administered by continuous infusion in small doses, raises the sodium output in both histamine- and gastrin-stimulated juices. Atropine reduces it. The reciprocal relationship of $[H]$ and $[Na]$, however, is not altered.

(ii) A rise in the output of sodium occurs during the first

Subcutaneous dose of stimulant	Sodium Output mEq/First hour
Gastrin 2 ug/kg + Mecothane 5 mg	5.7
Gastrin 2 ug/kg	5.5
Gastrin 4 ug/kg	4.3
Gastrin 0.5 ug/kg	3.0
Histamine 40 ug/kg + Gastrin 2 ug/kg	3.0
Histamine 40 ug/kg	2.6
Histamine 40 ug/kg + Mecothane 5 mg	5.7
Gastrin 2 ug/kg + Atropine 0.6 mg	1.8

TABLE IV. The sodium output of subject G. M. during the hour following subcutaneous administration of stimulants singly or in combination.

10 to 20-minute period following all modes of administration of secretory stimulants. This is followed by a rapid fall to low levels during most of the experimental period, with a slight tendency to rise again towards the end. The extent of this initial rise and subsequent fall in output are in general agreement with the results reported in animals [103].

(iii) The output of sodium during the first 20-minute period following single intravenous injections of gastrin appears to depend on the level of the dose (Fig. 7). In the subsequent 20-minute period, and independently of the dose, the sodium output falls to levels similar to those obtained during spontaneous secretion prior to the test.

D. Potassium Secretion

The present series of experiments offered the opportunity of investigating the pattern of potassium secretion following administration of various stimulants, singly or in combination. In every case, including that of gastrin, a peak of $[K]$ preceded that of $[H]$ (Fig. 8).

It was noted in section I that when a steady-state of secretion is established by continuous infusion of histamine or gastrin, $[K]$ falls from an early peak to a steady plateau which correlates closely with secretory rate and $[H]$.

Following single intravenous injection of gastrin, however, when the secretory rate changes rapidly, $[K]$ rises to an early peak and falls rapidly to levels below those obtained during spontaneous secretion/

secretion (Fig. 6).

The peak output of potassium following continuous infusion or single intravenous injections of gastrin is like that of acid, distinctly dose-dependent (Fig. 9). The slope of the sigmoid curve relating the logarithm of the dose and peak output is similar for both acid and potassium, and is a further indication that potassium in the juice is mainly of parietal origin.

E. Relationship of Hydrogen Ion Concentration to the Concentration of Sodium, Potassium and Chloride.

The theoretical statement of the two-component hypothesis proposed in section I is further extended to account for the observed variations in ionic concentration under all conditions of stimulation.

The symbols retain their meaning. It will be recalled that the primary parietal concentrations (H_o, K_p, Cl_p) and the primary non-parietal concentrations (Na_o, Knp, $Clnp$, b) are considered constant.

For any single specimen, Eqs. b.3, c.2, d.2, and e.1 can be transformed respectively into the following:

from Eq. b.3 $$k/v = \frac{H_o - [H]}{H_o + b}$$ Eq. g.1

from Eq. c.2 $$k/v = \frac{[Na]}{Na_o}$$ Eq. g.2

from Eq. d.2 $$k/v = \frac{K_p - [K]}{K_p - Knp}$$ Eq. g.3

from/

$$\text{from Eq. e.1} \qquad k/v = \frac{Ho + Kp - [Cl]}{Ho + Kp - Nao - Knp + b} \qquad \text{Eq. g.4}$$

It should be emphasised in this context that k is not a constant but represents simply the volume of non-parietal component in a particular sample.

From Eqs. b.3, c.2, d.2, and e.1, and Eqs. g.1 - g.4, it can be concluded that the concentration of an ion in any single specimen is dependent on the ratio of k/v, that is, on the ratio of the non-parietal volume to total volume and, by implication, of non-parietal to parietal volume. As was clearly demonstrated in the first section, the non-parietal volume during steady-state secretion is constant, as indicated by the constant output of sodium per specimen. Under these conditions, the concentration of an ion is linearly related to 1/v, which, besides the observed concentration, is the only other variable in the equations.

Eqs. g.1 - g.4 can further be extended to account for the linear relationship observed under all conditions between the concentration of an ion and that of another

(i) Combining Eqs. g.1 and g.2, it can be shown that

$$Na = \frac{Nao(Ho - [H])}{Ho + b} \qquad \text{Eq. g.4}$$

Since the primary concentrations H_o, Na_o and b are constant, the relationship of $[H]$ and $[Na]$ is linear

$$\text{At } [H] = -b, \quad [Na] = Nao$$

$$\text{At } [H] = Ho, \quad [Na] = 0$$

(ii)/

(ii) Combining Eqs. g.1 and g.3, it can be shown that

$$[K] = K_p - \frac{(K_p - K_{np})\,(H_o - [H])}{H_o + b} \qquad \text{Eq. g.5}$$

from which it can be deduced that the relationship of H and K is linear.

At $[H] = H_o$, $[K] = K_p$

At $[H] = -b$, $[K] = K_{np}$

(iii) It can be similarly be shown by combining Eqs. g.1 and g.4 that

$$[Cl] = K_p + H_o - \frac{(H_o - [H])\,(H_o + K_p + b - Na_o - K_{np})}{H_o + b} \qquad \text{Eq. g.6}$$

from which it can be deduced that the relationship of H and Cl is linear.

At $[H] = H_o$, $[Cl] = H_o + K_p$

i.e. the sum of the primary parietal potassium and hydrogen ion concentrations.

At $[H] = -b$, the sum of the non-parietal cations equals the sum of the non-parietal anions:

$$Cl_{np} + b = Na_o + K_{np}$$

It is clear from Eqs. g.4 - g.6 that the intercepts at H_o provide/

No. of Samples	Volume/20'	[H]	[Na]	[K]	Cations	[Cl]	Na Output/20'	P/NP	Calculated Volume
6	10.8	15.1	106.3	7.2	128.6	129.6	1.148	0.296	10.9
19	10.5	32.7	91.3	10.4	134.4	133.8	0.958	0.494	10.5
17	17.0	50.0	78.0	12.5	140.5	141.5	1.326	0.753	17.0
12	20.7	69.8	61.3	12.4	143.5	144.5	1.269	1.207	20.5
8	33.0	90.5	44.3	13.6	148.4	149.8	1.462	2.034	32.4
37	41.6	112.8	28.1	13.9	154.8	154.2	1.169	3.836	41.3
63	61.3	129.4	16.4	16.3	162.1	161.7	1.005	7.389	61.6
8	70.7	142.8	6.4	17.4	166.6	167.6	0.452	20.586	71.4

TABLE III. The mean secretory rate per 20 minutes and corresponding ionic concentrations. The last two columns represent the ratio of parietal to non-parietal volume and the calculated volume. For details, see text.

provide estimates of the primary parietal concentrations, while the intercepts at -b provide estimates of the primary non-parietal concentrations.

Analysis of the results:

Out of a total of 170 samples from subject GM, 56 had been obtained following single intravenous injections of gastrin, and a further 29 samples during steady-state secretion induced by continuous infusion of gastrin.

A regression analysis of the linear relationship between hydrogen ion concentration and the concentrations of Na, K, Na + K, and Cl were performed as follows:

(i) A complete regression analysis was performed on each of the 170 samples (Table II, column A).

(ii) These same samples were also grouped into 8 fractions, each of which corresponded to two decades of acidity (0 - 20, 20 - 40 , etc.) and a linear regression performed.

(iii) The 170 samples were weighted each for its volume and grouped into 8 fractions, as in (ii) above, for regression analysis (Table II, column B). The concentration levels of these fractions were almost identical to those obtained in (ii) and are given in Table III. The rise of $[H]$, $[K]$ and $[Cl]$ and the fall of $[Na]$ with secretory rate is evident.

(iv) A separate regression analysis was also performed on each of the 56 samples obtained following single intravenous injections/

	A	B	C	D	E
H_o	148.9 ± 0.5	149.4 ± 0.9	147.6 ± 0.8	148.4 ± 1.4	147.9 ± 2.3
K_p	16.9 ± 0.3	17.5 ± 0.7	14.7 ± 2.0	16.8 ± 0.9	16.9 ± 0.5
$H_o + K_p$	165.8	166.9	162.8	165.9	164.8
Cl_p	166.3 ± 0.5	167.2 ± 0.5	163.3 ± 1.1	164.6 ± 0.9	164.4 ± 0.8
Na_o	136.7 ± 0.9	136.4 ± 1.0	139.2 ± 1.3	140.0 ± 1.6	138.6 ± 3.9
Knp	6.4 ± 0.6	6.0 ± 0.9	5.2 ± 3.9	5.5 ± 1.2	4.3 ± 1.1
Clnp	117.0 ± 0.9	118.1 ± 0.8	116.8 ± 2.1	121.9 ± 1.3	121.8 ± 0.5
b	25	25	25	25	25
Clnp + b	142.0	143.1	141.8	146.9	146.8
Na_o + Knp	143.1	142.4	142.4	145.5	146.8
Na^+ vs H^+	r = −.994	r = −.999	r = −.995	r = −.999	1/v vs H^+ r = −.996
K^+ vs H^+	r = +.776	r = +.956	r = +.60	r = +.906	1/v vs Na^+ r = +.995
Cl^- vs H^+	r = +.947	r = +.998	r = +.976	r = +.992	1/v vs K^+ r = −.970
$K^+ + Na^+$ vs H^+	r = −.996	r = −.996	r = −.986	r = −.997	1/v vs Cl^- r = −.993

Table II. The calculated primary concentrations in the parietal and non-parietal components with their standard errors.

injections of gastrin (Table II, column C).

(v) A further regression analysis was performed on the 29 samples obtained during continuous infusion of gastrin (Table II, column D). The intercept of the regression of [Na] on [H] (x-axis) gives the value of H_o. From this value and the regression of [K] and [Cl] on [H], the value of K_p and Cl_p can be obtained (Fig. 10).

Repeated plasma bicarbonate estimations on subject GM gave a value of 25 mEq/L $\pm$ 1. Assuming a value for b, analogous to that of plasma bicarbonate, estimates of the primary non-parietal concentrations Na_o, K_{np} and Cl_{np} can be obtained (Fig. 10).

An inspection of the results in Table II permits of the following deductions:-

(a) Relationship of primary concentration and stimulus

The close similarity of the values obtained from the various regression equations both for the gastrin data alone (columns C, D, E) and for all the data combined (columns A, B) shows that the primary concentrations of the two components are independent of the stimulant, or combination of stimulants, or mode of administration.

(b) Relationship between the concentration of the various ions.

Since the factor k/v does not appear in Eqs. g.4 - g.6, the linear relationship between [H], [Na], [K] and [Cl] is independent of secretory rate and time of collection. Thus the main series

was/

was made up variously of 5, 10, and 20-minute samples, while the intravenous gastrin series was made up of 5-minute samples exclusively.

(c) The constancy of the primary concentrations.

While the intravenous gastrin samples were obtained over the last six of the twenty-month period of testing, those following continuous infusion of gastrin had been obtained during the first twelve months. The close similarity of the values for the primary concentrations in those two series argues in favour of the overall constancy in composition of the two components in a particular subject.

(d) Relationship of concentration and secretory rate.

While as noted above in (b) the relation of the concentration of an ion to that of another is independent of secretory rate, the actual concentration achieved by an ion in a particular sample is strictly dependent on the ratio of k/v, that is, on the ratio of non-parietal to total volume and, by implication, on the ratio of non-parietal to parietal volume (Eqs. g.1 - g.4).

From the output of acid and sodium in a sample and the value of the primary concentrations H_o, Na_o and b, the volume of non-parietal and parietal components can be calculated. Their sum should be equal to the observed volume (Table III), and their ratio condition the concentration level achieved by an ion. This latter prediction is verified by calculating this ratio for the samples obtained following single intravenous injections of gastrin and plotting/

plotting it against the observed [H] and [Na] (Fig. 11).

It was noted in section I that under steady-state conditions, when a constant non-parietal volume k is produced, the relationship of secretory rate and concentration is hyperbolic, while that of 1/v and concentration is linear. This is further verified by the following transformations of data obtained mostly under non-steady-state conditions.

Firstly, by assuming a sodium output identical to that obtained following infusion of gastrin, namely, 0.813 mEq/20 mins., to be present in each of the fractions in Table III, it is possible to re-calculate the expected secretory rate and concentrations. The curve shown in Fig. 12 is the same as that derived from the infusion data shown in Fig. 4, section I. It is clear from the fit of the re-calculated data to the same curve that ionic concentration is accurately and predictably dependent on secretory rate in the presence of a constant non-parietal component or sodium output.

Secondly, the data obtained during non-steady secretion following single intravenous injections of gastrin were arranged into series, each made up of samples possessing a similar sodium output within a narrow range. A series of hyperbolae relating [H] and [Na] to secretory rate can be drawn (Fig. 13). As expected, the intercept on the volume axis varies with the sodium output of the series, while all the asymptotes merge towards the primary acidity. Since each series is made up of a nearly-constant sodium output, the contribution of the non-parietal volume k is now a constant for each/

each series. Under these conditions, the relationship of concentration to 1/v in each series is linear (Fig. 14).

Discussion.

1. The relationship of secretory rate and ionic concentration.

The importance of the sodium output, in the absence of contamination, as the pure index of the non-parietal component has not been sufficiently recognised. A closer study of this inorganic constituent provides the clue to the relationship of secretory rate and ionic concentration in gastric juice.

The overall constancy of Na_o, the primary non-parietal sodium concentration, is evident from its calculated value under all modes of stimulation (Table II). The sodium output, i.e. the product of k, the non-parietal volume and $[Na]$, may vary. During steady-state secretion, the output of sodium is constant, which leads to the conclusion that k, the volume in which it appears, is also constant. Under these conditions, the relationship of ionic concentration and secretory rate v is hyperbolic, while that of ionic concentration and 1/v is linear (Eqs. b.3, c.2, d.2, e.1, section I).

During non-steady-state secretion, however, the output of sodium is not constant but varies with the nature and dose of stimulant, or combination of stimulants, and the time following stimulation. Although the primary concentration of sodium remains unaltered, the volume of the non-parietal component and, therefore, the output of sodium, vary. Under these conditions, ionic concentration/

concentration is dependent on the ratio of k/v (Eqs. g.1 - g.4, Fig. 11). If, however, the sodium output is assumed to be fixed, so that k, the non-parietal volume, is now constant, and the data re-calculated, the strict relationship between ionic concentration and secretory rate re-appears clearly (Fig. 12).

The well-known effect of cholinergic agents which increase the sodium output, or anti-cholinergic agents which depress it, in dissociating the relationship of secretory rate and concentration is attributable to their unequal effects on the parietal and non-parietal volume outputs.

2. Relationship between the concentration of various ions.

Since the factor k/v, or volume factor, disappears from Eqs. g.4 - g.6, it is clear that the linear relationship which the concentration of an ion bears to that of another is independent of secretory rate, and should therefore hold for both steady-state and non-steady-state secretion. This is confirmed by the results in Table II.

The curvilinear relationship, theoretically predictable on the basis of the back-diffusion hypothesis [104] between [H] and [Na], has not been confirmed by this study. This is the more significant since the data presented span the entire range of concentration levels. In human gastric secretory studies, the possibility of salivary contamination is ever present and may have been, at least in part, responsible for the slight curvilinearity shown in some reported data in man. The method used in this study appears to have ensured virtual elimination of this potential source of error,

as/

as indicated by the high [Na] and [Cl] in the presence of the lowest [H] and [K].

A similar analysis of interionic concentrations was performed on electrolyte data communicated to us and obtained by Dr. Nordgren during steady-state secretion induced by continuous infusion of histamine in five human subjects [98]. The relationship of [Na] and [H] for all subjects is clearly linear, with a high coefficient of correlation ($r = 0.987$). The acidity intercept gives a value of 148.1 mEq/L, which is almost identical with the value obtained in subject GM. A separate regression analysis of [Na] and [H] in one subject (H.C.), whose data span adequately the line, gives the following values: $r = 0.984$; $H_o = 149.7$; $Na_o = 135.6$. Again the close similarity with the data in Table II is evident.

3. Under steady-state conditions, a precise relationship with a high degree of correlation (Table II, columns D, E) exists between [K] and secretory rate; [K] and [H]; potassium output and acid output.

A more abrupt rise and precipitate fall in [K] as compared with [H] is evident, however, under non-steady-state conditions induced by subcutaneous or intravenous administration of secretory stimulants, including gastrin. For this reason, the correlation between [K] and [H] under non-steady-state conditions, though distinct, is not as high as for other ions. A higher correlation can be obtained if the data during the initial rise of concentration are plotted separately. By comparison with the infusion data, these deviations

of/

of [K] from those expected are small, rarely exceeding 2 mEq/L. The calculated primary potassium concentrations for the whole series are closely similar to those obtained following infusion. The small deviations of [K] are especially evident at the higher acidities and impart, in this range, a slight upward shift to the plot of [Cl] versus [H].

The explanation of the behaviour of [K] under non-steady-state conditions is still obscure. The similarity to potassium transients in salivary secretion has been noted by a number of observers [78]. A transient rise in plasma [K] has been observed in some species [105] following administration of histamine. This has not been confirmed in either man or dog by Nordgren [98]. The blood samples, however, were examined during steady-state secretion so that an earlier transient rise cannot be excluded.

The patterns of secretion of potassium in gastrin- and histamine-stimulated juices are similar. This is to be expected in view of the strong suggestive evidence in favour of the release of parietal intracellular histamine by gastrin [61]. A specific action of histamine on the permeability of frog gastrin mucosa to potassium has been reported by Villegas [106]. Whether the observed changes in [K] are to be attributed to a specific action of histamine on gastric mucosa or to a physico-chemical process connected with the "rate of change" of secretory rate mostly evident in non-steady states, awaits further study.

4. It has been noted that the sodium output varies with the stimulant and its mode of administration. In the first 20 minutes

of/

N-P Component	MAN Makhlouf et al.	DOG Altamirano	DOG Hollander
Na^+	136.7	137.8	133.0
K^+	6.4	3.5	4.4
Cl^-	117.0	116.6	120.0
HCO_3^-	25	24.2	25.9*

Table V.

of a secretory period following intravenous injection of gastrin, the output appears to be dose-dependent. The interpretation of these findings and a further discussion of the factors controlling the production of the non-parietal component will be undertaken in section III.

By topical application of acetylcholine to the vagotomised pouches of antrectomised dogs, Hollander [107] has obtained a cell-free alkaline fluid of analogous composition to interstitial fluid. More recently, Altamirano [108] has obtained similar results by gastric intra-arterial infusion of acetylcholine. The data obtained in this study on man show that the chemical composition of the non-parietal component in man is closely similar to that found by these two workers in dogs(Table V).

Repeated estimations of plasma electrolytes in subject GM show little change. Assuming a Donnan equilibrium to operate and expressing the data in mEq/kg H_2O, the product of plasma sodium and chloride concentrations is almost identical with the product of sodium and chloride in the non-parietal component. This is to be expected if, as it is proposed, the non-parietal component is essentially gastric interstitial fluid.

Summary

1. The two-component hypothesis is further extended to account for the observed variations in ionic concentrations and secretory rate under non-steady-state conditions of secretion.

2. A linear relationship, independent of stimulant or mode of stimulation/

stimulation, is shown to exist under all conditions of secretion between the concentrations of the various ions.

3. A distinct relationship of ionic concentration to secretory rate is demonstrated and shown to depend on the relative proportion of non-parietal component in a specimen. The importance of the sodium output as a precise index of the non-parietal component is established.

4. The calculated composition of the non-parietal component shows it to be identical with interstitial fluid.

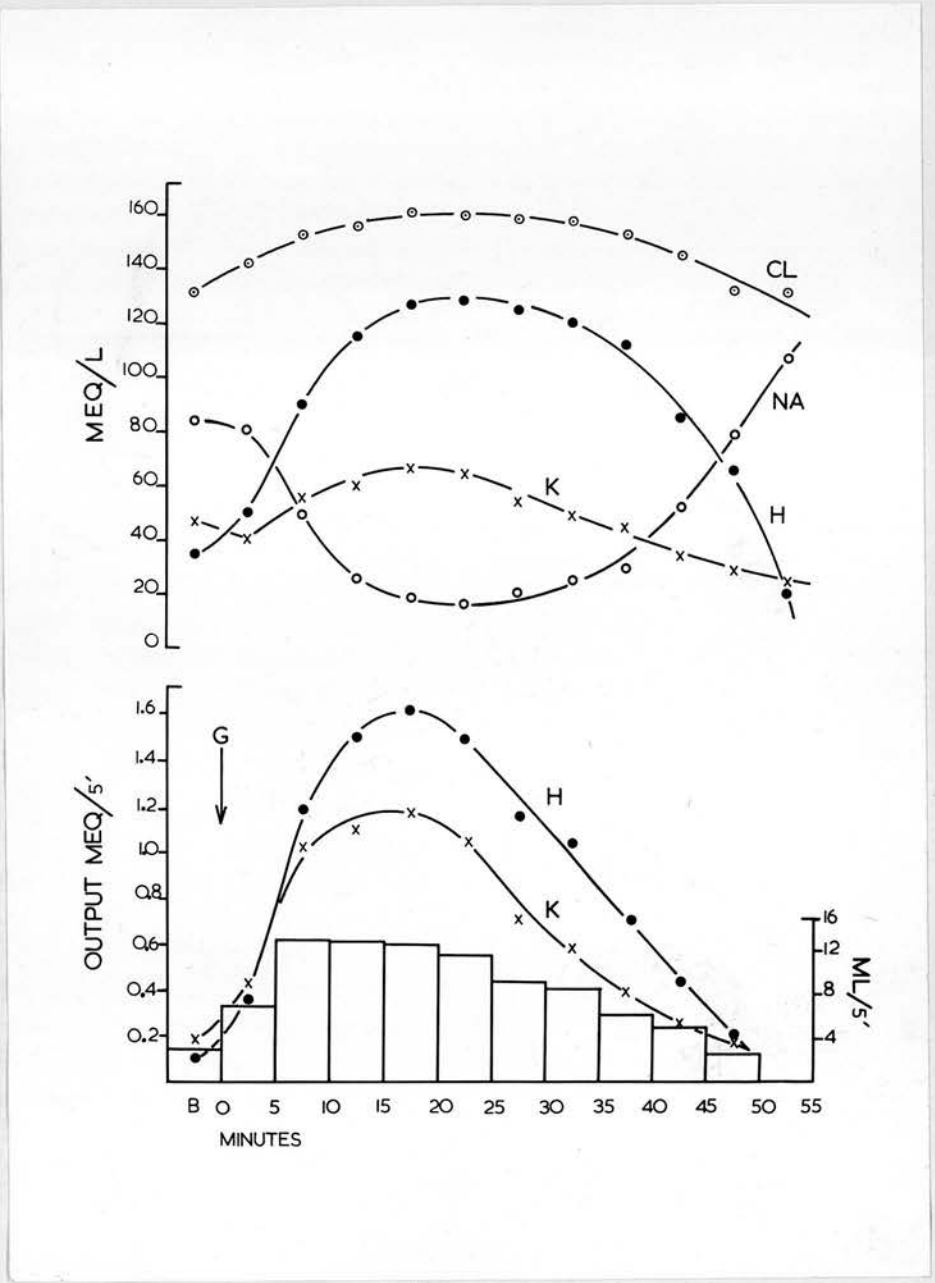


Figure 6: The pattern of secretion following intravenous injection of 4 ug. of gastrin II in subject GM. The scale of potassium output has been increased tenfold and that of potassium concentration fourfold.

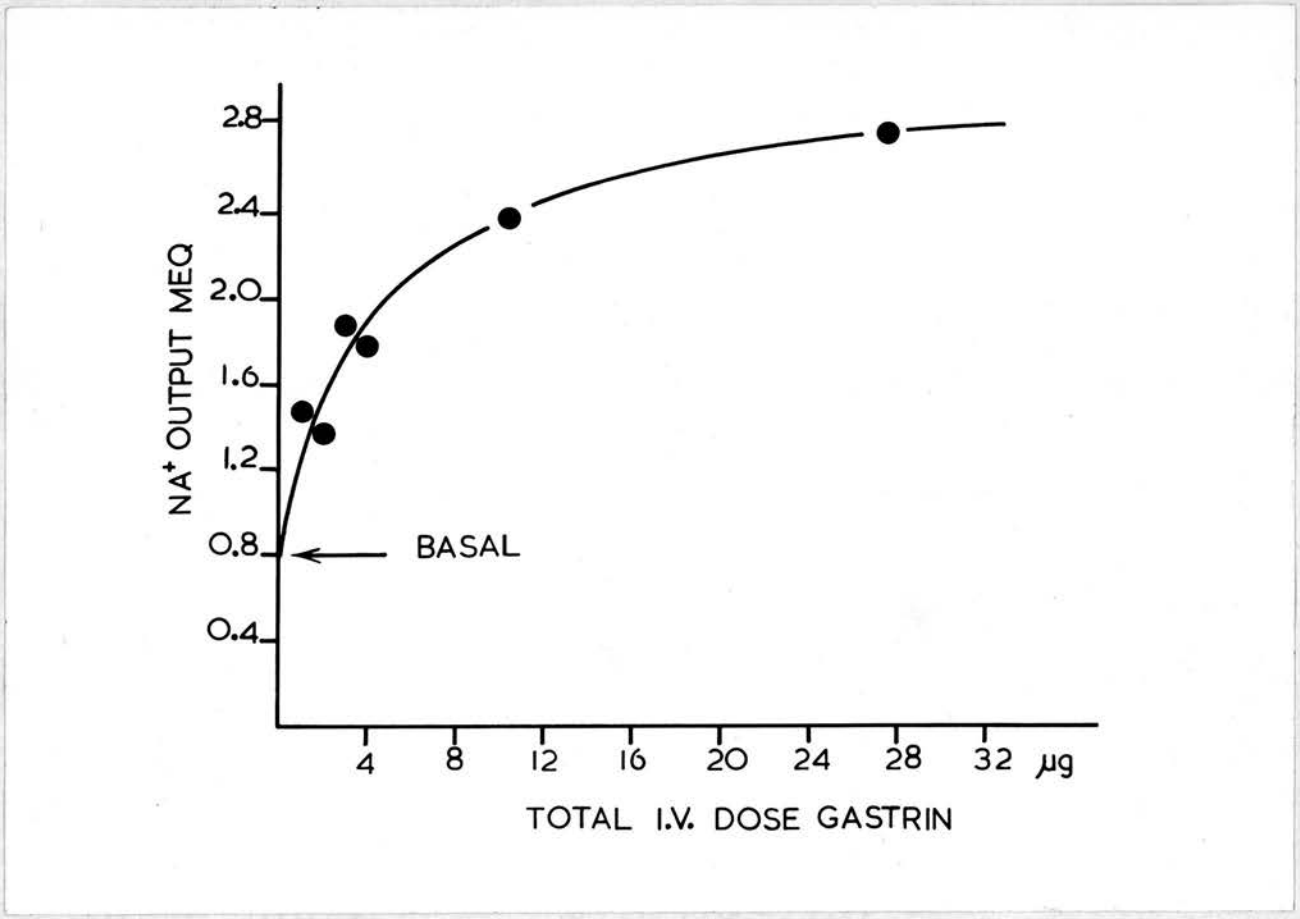


Figure 7: The sodium output in the first 20 minutes following intravenous injections of gastrin II in subject GM.

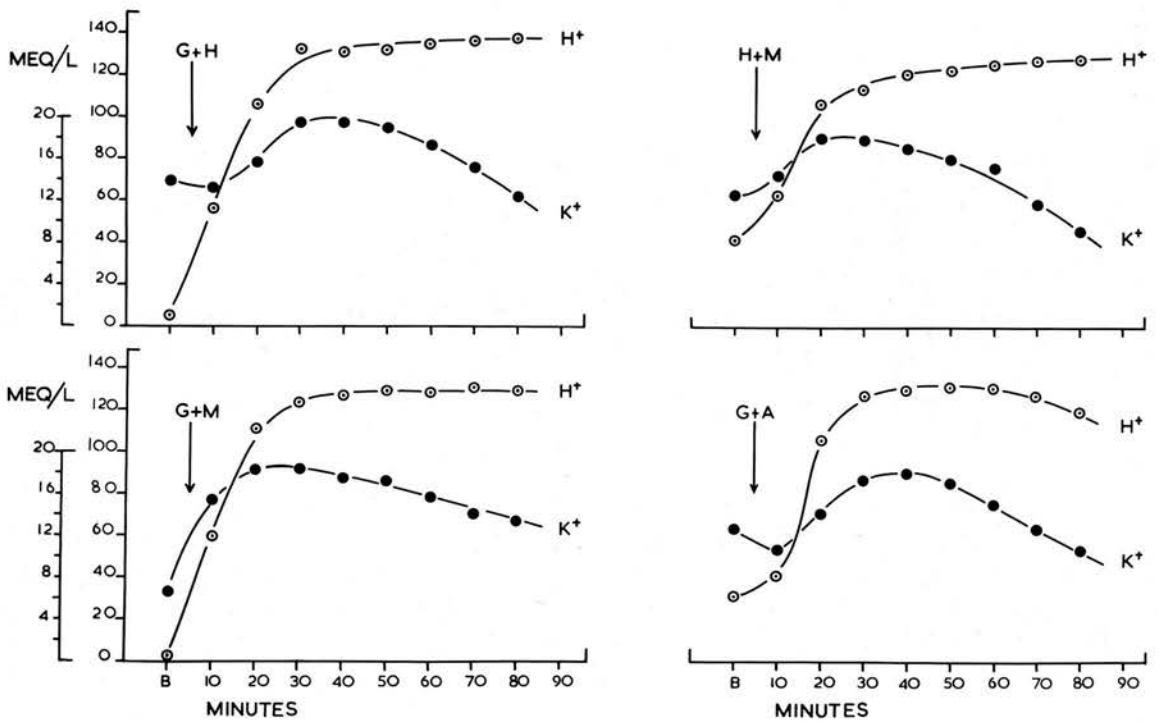


Figure 8: The earlier peak and more rapid fall of [K] as compared with [H], following subcutaneous administration of gastrin plus mechothane (G + M), gastrin plus histamine (G + H), gastrin plus atropine (G + A), and histamine plus mechothane (H + M). The upper two figures are from subject JM and the lower two from subject GM.

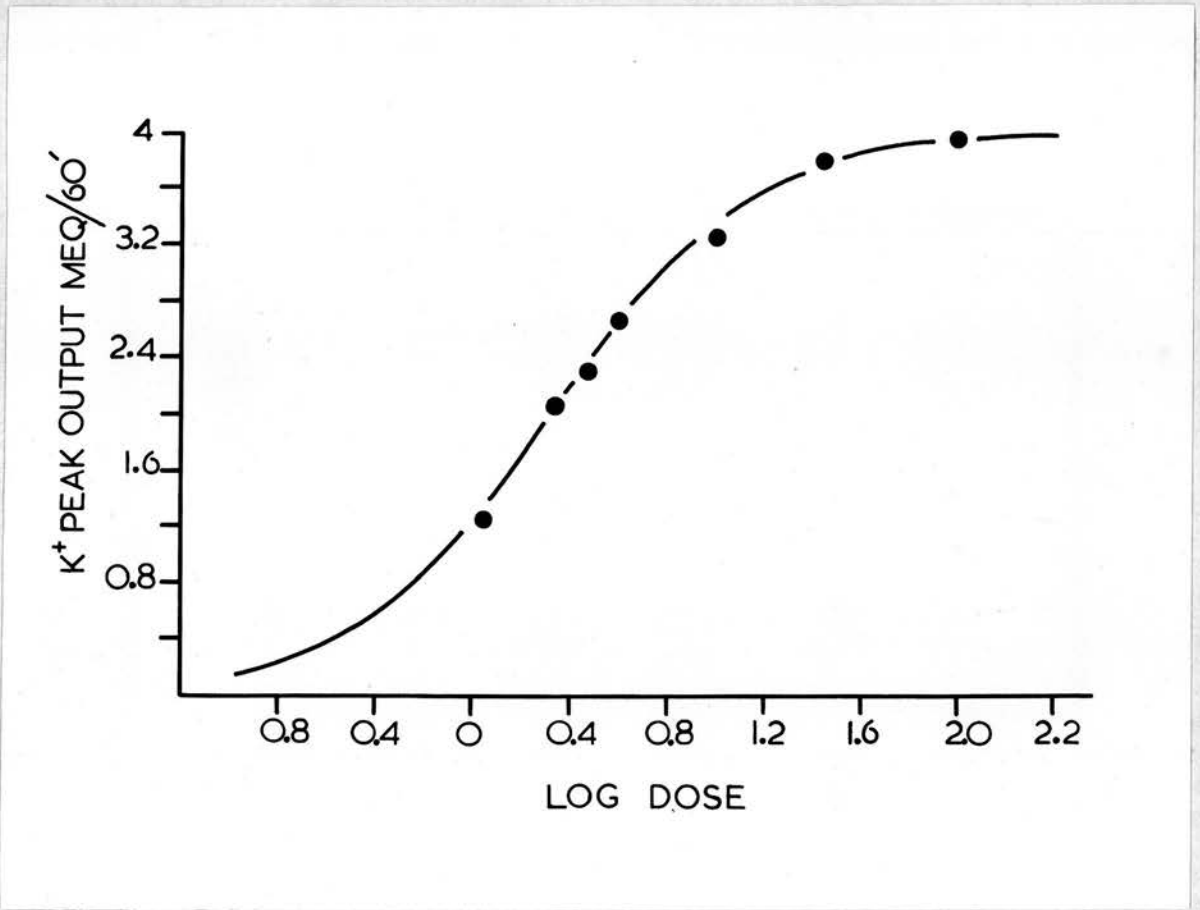


Figure 9: The relationship of the peak output of potassium following single intravenous injections of gastrin II in subject GM to the logarithm of the dose of gastrin.

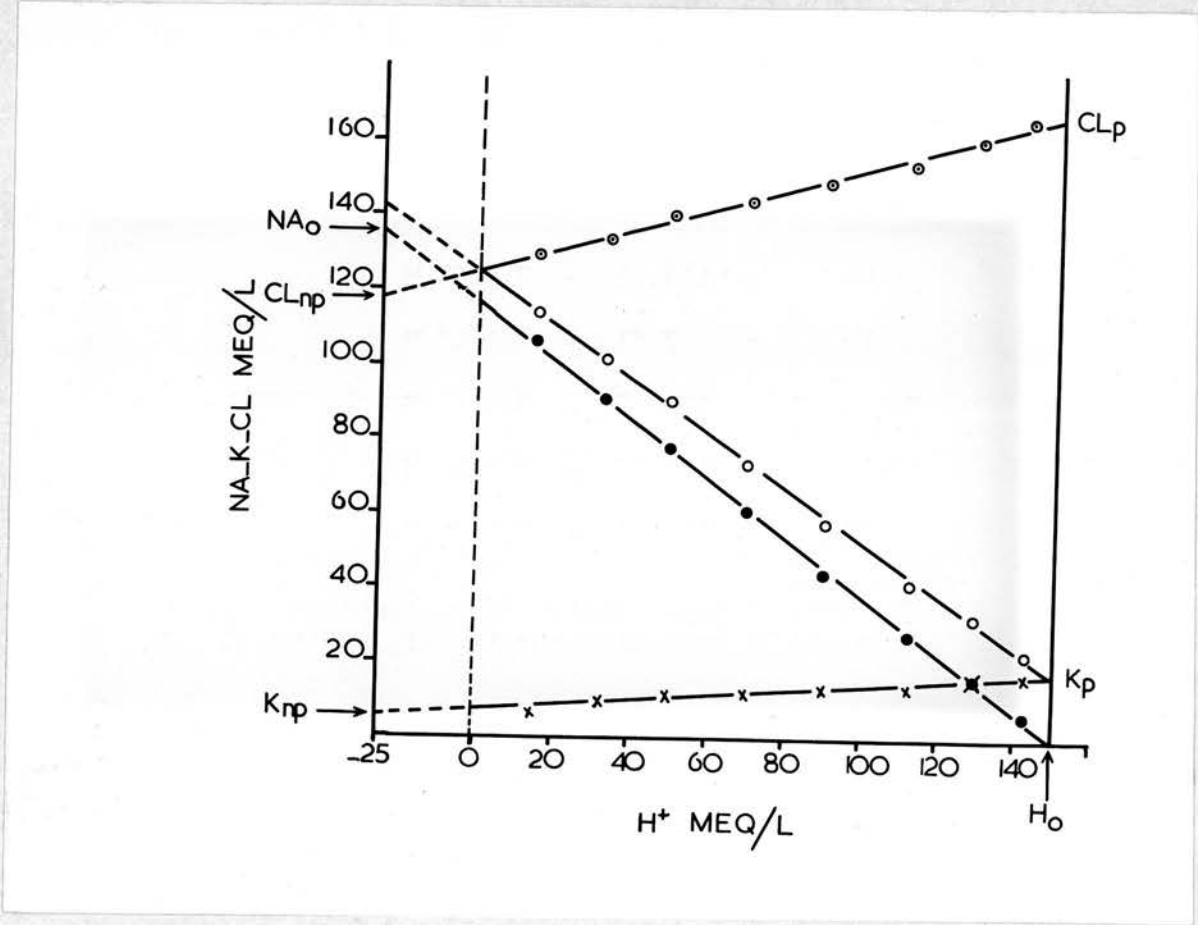


Figure 10: The linear relationship of [H] to [Cl], [Na], [K] and [Na] + [K]. The intercepts indicate the values of the primary parietal and non-parietal concentrations.

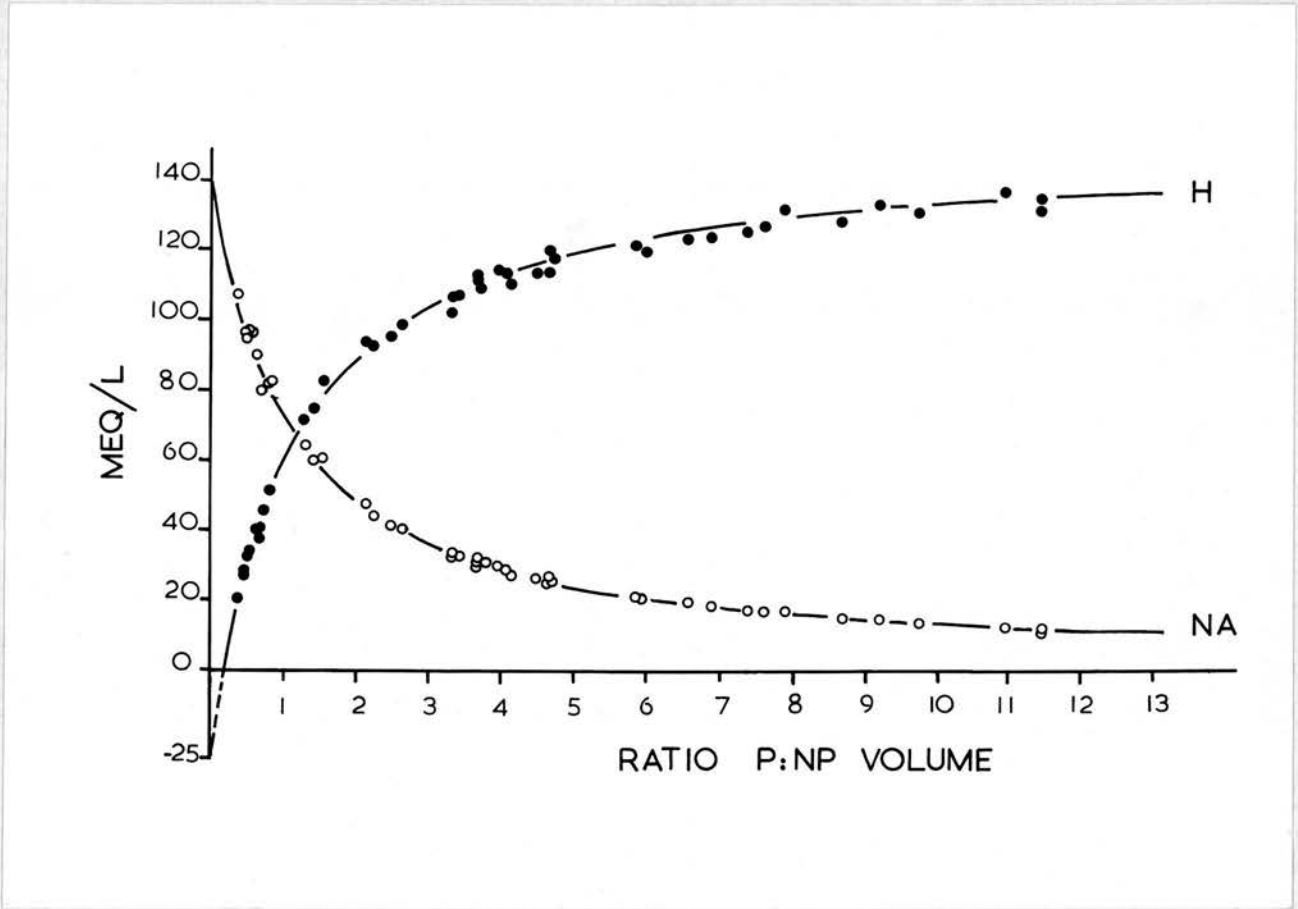


Figure 11: The relationship of [H] and [Na] to the ratio of the calculated parietal (P) and non-parietal (NP) volumes. The points represent the observed concentrations in five-minute samples obtained following single intravenous injections of gastrin II in subject GM.

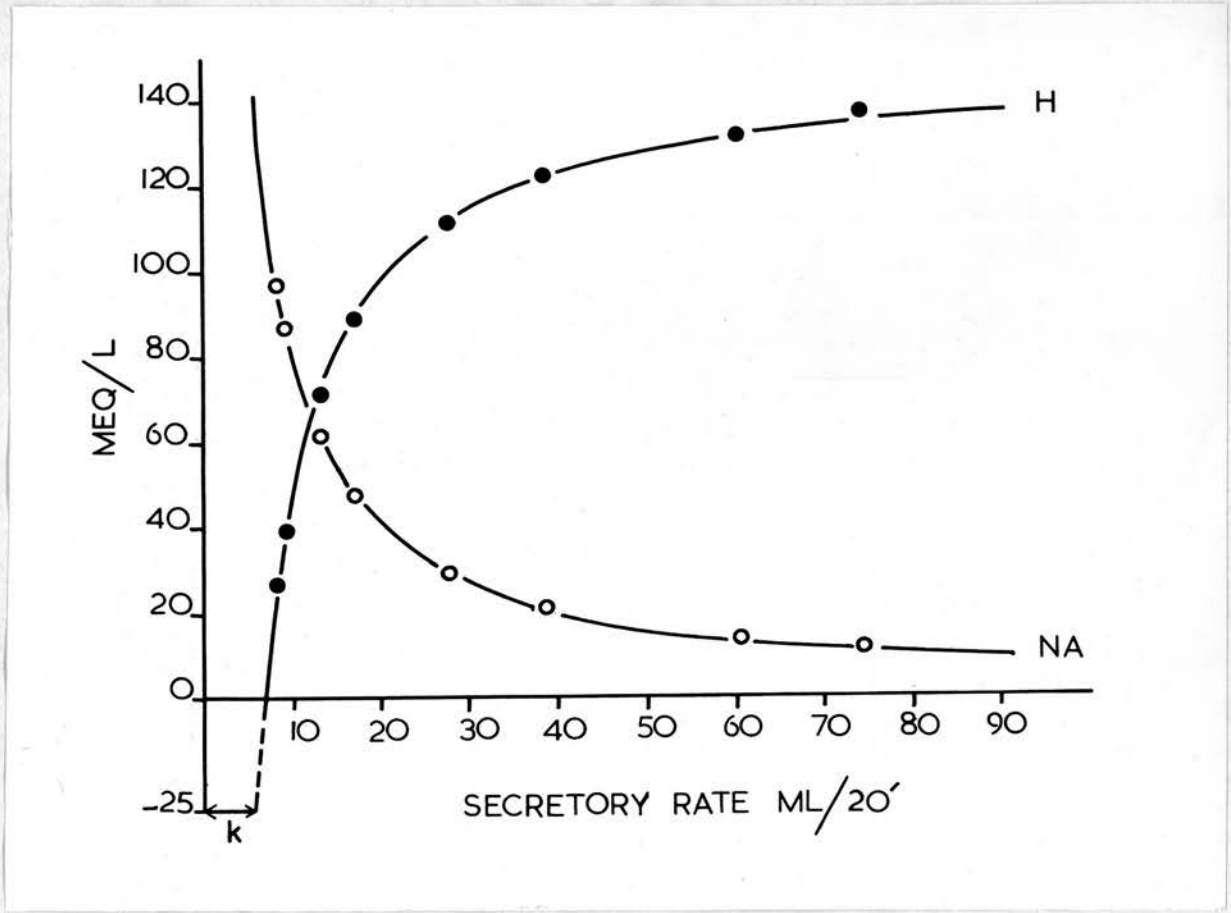


Figure 12: The fit of the re-calculated data from Table III to the curves from Fig. 4.

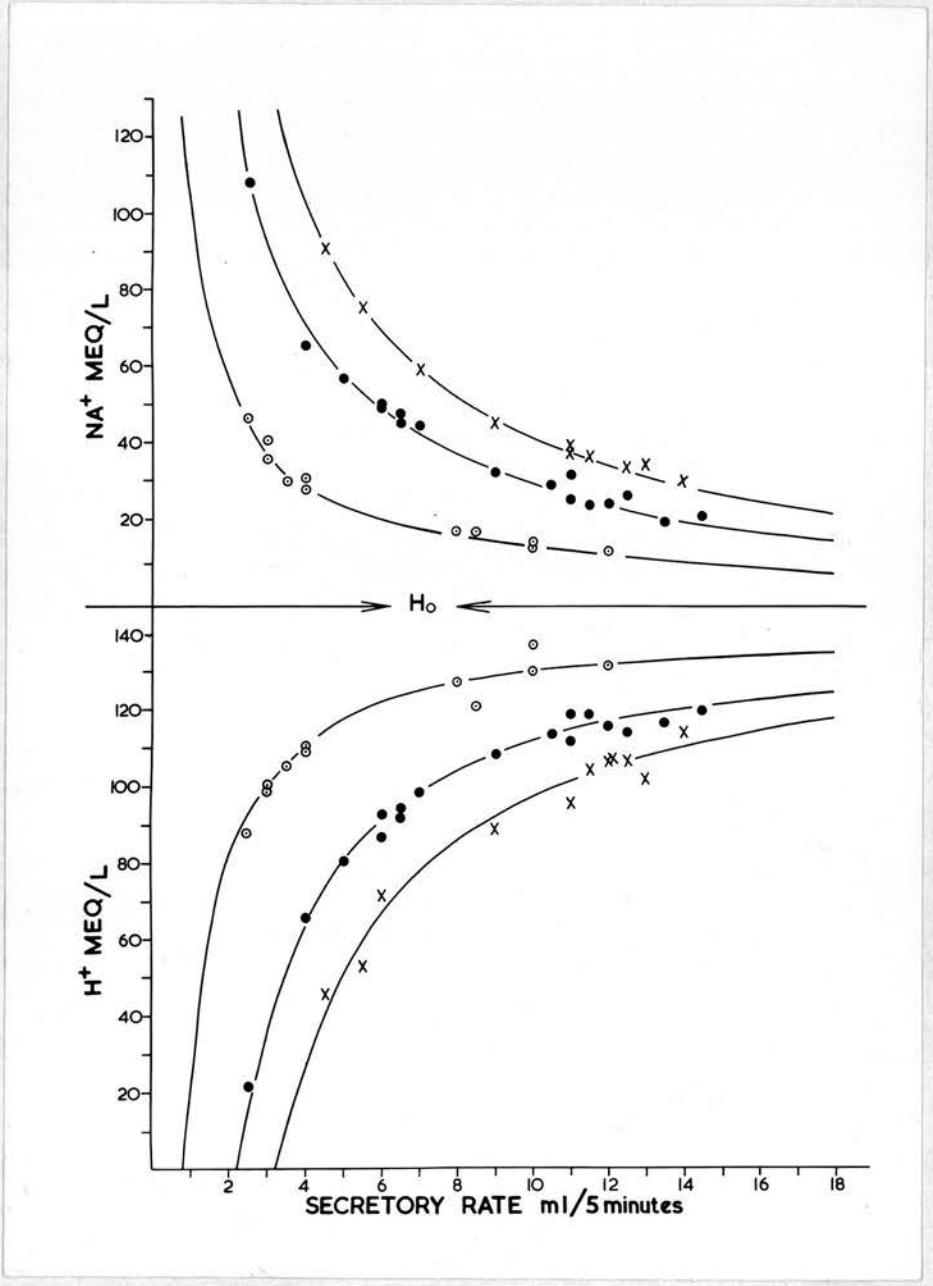


Figure 13: The relationship of secretory rate to [H] and [Na] following single intravenous injections of gastrin II in subject GM. The points in each series represent five-minute samples with closely similar outputs of sodium.

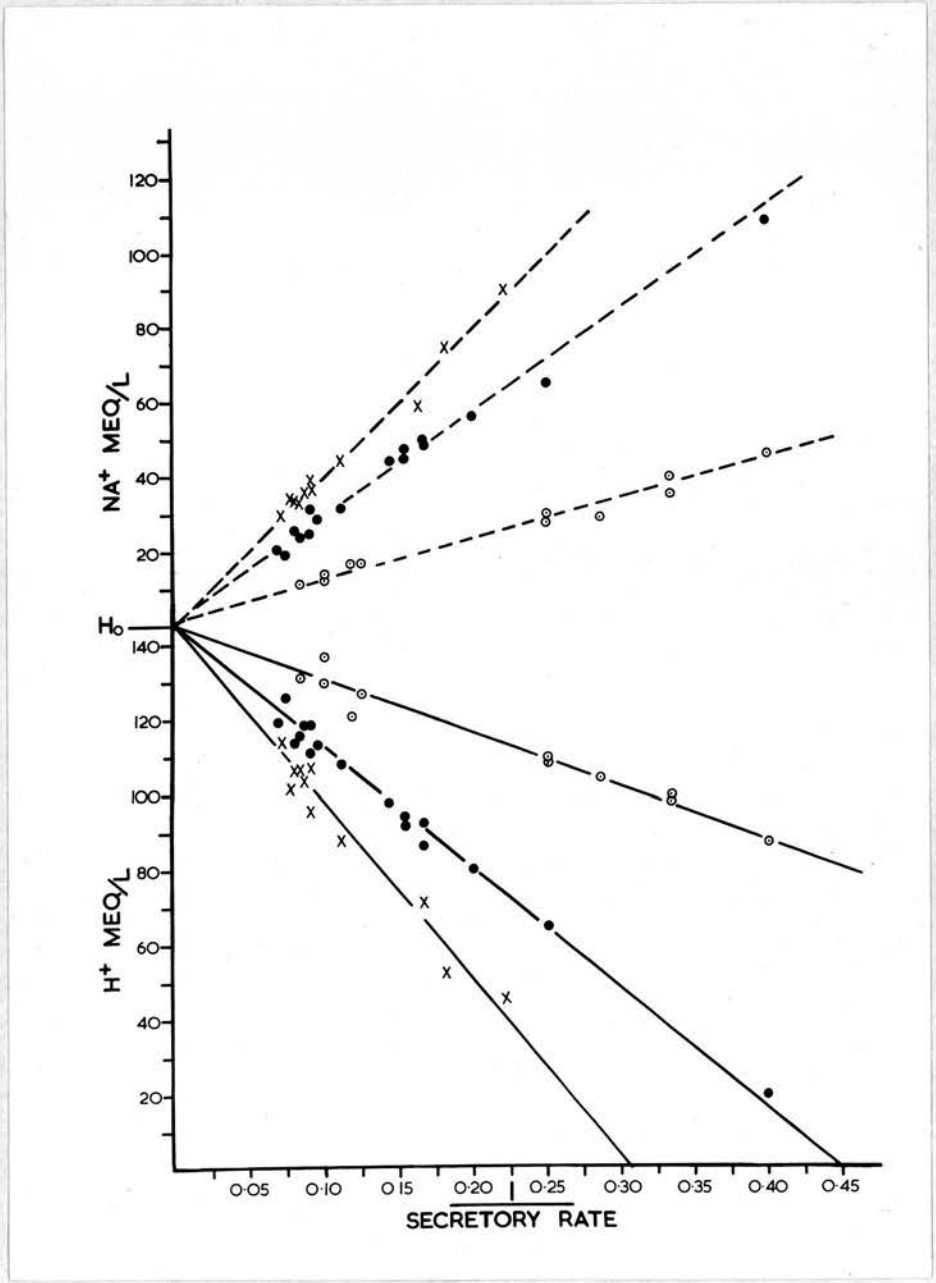


Figure 14: The linear relationship of 1/v to [H] and [Na] following single intravenous injections of gastrin II in subject GM. The series was the same as those shown in Fig. 13.

Section III

Implications of the Two-Component Hypothesis

Gastric juice may be considered as a mixture, in varying proportions, of two primary components, in which diverse cellular products, such as pepsin, visible and dissolved mucus, intrinsic factor, etc., are dispersed, and to which they contribute negligibly, if at all, in volume and electrolyte content. The parietal cells are responsible for an active, slightly hyperosmotic, secretion of HCl and KCl, while the non-parietal component is probably of extracellular origin, identical in composition with interstitial fluid and somewhat hypo-osmotic to plasma.

There is fairly general agreement as to the composition of parietal secretion. The steady-state concentration of its chloride, both in man and dog, is around 166 mEq/L [98]. The parallelism of acid and potassium secretion is evident under steady-state conditions. The dependence of both acid and potassium concentration on secretory rate, and of acid and potassium output on dose of stimulant, indicates that the parietal secretion contains both these cations (sections I and II). Their relative concentrations, however, are different in the two species, with a lower acid and a higher potassium concentration in man [98].

The evidence for the composition of the non-parietal component has in general been obtained in two main ways:-

(i) Analysis of the relationship to acidity, of ions thought to originate exclusively in this component, namely, sodium and calcium/

calcium.

(ii) Analysis of the electrolyte composition of this component collected in the "pure" state.

A major difficulty in (i) has been the value that should be assigned to the neutralising bicarbonate radical in this component. Fisher and Hunt [95] in man, and Gray and Bucher [97] in dogs, have sought to circumvent this by assuming that the two components were iso-osmotic. Whereas a great deal of evidence has accumulated showing that parietal secretion is somewhat hyperosmotic to plasma [57, 109], all the evidence from direct estimation suggests that the osmolality of the non-parietal component is analogous to that of interstitial fluid and marginally less than that of plasma [107, 108]. It is of interest to note in this connection that, when a value analogous to that in the plasma or interstitial fluid is attributed to the bicarbonate in the non-parietal component, the calculated levels for sodium, potassium and chloride concentrations are those predicated for these ions in the interstitial fluid (sections I and II).

Possible Objections to the Two-Component Hypothesis.

(a) An important postulate of the back-diffusion hypothesis of acidity regulation is that the gastric mucosa is permeable to back-diffusion of hydrogen ions, as well as to entry of sodium and other extra-cellular ions, with the singular exception of bicarbonate [13]. Recent evidence, however, suggests that the gastric mucosa, unless damaged, is virtually impermeable to back-diffusion of anything but minute amounts of hydrogen ions [110]. On the other hand/

hand, a number of workers have demonstrated conclusively that bicarbonate does penetrate the lumen of the canine stomach. Bicarbonate was found by Hollander [107] in the anacid resting juice of vagotomised canine pouches and also following topical application of acetylcholine. More recently, Altamirano [108], using a chambered preparation of canine gastric mucosa with intact blood supply, has demonstrated that gastric intra-arterial infusion of acetylcholine leads to the passage into the lumen of the stomach of an alkaline fluid in all respects analogous to gastric interstitial fluid. Changes imparted to the plasma were accompanied by parallel changes in the composition of the fluid entering the stomach.

Resting secretion is infrequently anacid in man except under pathological circumstances. The bicarbonate concentration in the spontaneous secretion of the normal subject JM occasionally reached 9 mEq/L. In patients with pernicious anaemia tested with subcutaneous gastrin II, the level of bicarbonate occasionally reached 15 mEq/L, the pH was alkaline, while the concentrations of sodium and potassium were very similar to their concentrations in plasma. The concentration of chloride, however, was somewhat higher (125 - 132) and varied reciprocally with the bicarbonate concentration. It is possible that under these and similar circumstances an exchange of bicarbonate and chloride may take place across the mucosa, altering the composition of the collected fluid.

(b) Instillation experiments have formed an important basis for the conclusions of the back-diffusion hypothesis. The postulate involved the exchange of hydrogen and sodium as ion pairs (HCl and NaCl)/

NaCl) without net movement of water. By the use of dilution indicators, however, Lindner et al. [111] have demonstrated a net addition of fluid into the lumen following instillation of acid solutions in man. Using a chambered preparation of canine gastric mucosa where volume changes could be accurately recorded, Bornstein et al. [112] could show a net movement of water and ions in the direction predicted by activity gradients across the mucosa. Thus a hypotonic solution of HCl (0.5 M) showed a significant loss of volume and hydrogen ions and a significant gain of chloride; while a solution of 0.15 M HCl showed a distinct increase in volume coincident with the dissipation of hydrogen ions. Similar considerations applied to the instillation of solutions of NaCl.

In the first two sections, it was shown that the predictions of the back-diffusion hypothesis regarding secretory rate and electrolyte concentration in man are not completely borne out by the experimental findings. A better fit of the data was obtained to the assumptions involved in a two-component hypothesis.

Mucus and Non-Parietal Component.

It is a misunderstanding of the two-component hypothesis to ascribe anything but a minute degree of acid-binding to the mucus in the juice. A number of early workers in this field showed an inverse relationship between hydrogen ion concentration and the concentrations of total nitrogen and sugar [113], while others have shown a direct relationship between the sodium and nitrogen outputs/

outputs [114]. However, not until the recent work of De Graef [115], elaborating on the findings of Glass [116], has the question been satisfactorily resolved. The essential constituents of the mucoprotein fraction of dissolved mucus in dogs appear to be pepsin and "glycoprotein". The near-constancy of composition of this fraction points to the synchronous functioning of the peptic and neck mucous cells presumed responsible for this product. The mucoproteose fraction, on the other hand, together with some polypeptides and non-protein-nitrogen, constitute for the most part the organic portion of the non-parietal component. An inverse linear relationship exists between the concentrations of each of the constituents of this mucoproteose fraction and acidity. The earlier work of Horowitz and Hollander [117] had shown that serum albumin constituted up to 44% of the total biuret-reacting protein of anacid mucus, but was present only in trace quantities in acid juice possessed of peptic activity.

Although it does not participate in electrolyte equilibria, this mucoproteose fraction, like the sodium output, appears to be an accurate index of the non-parietal component of the juice.

The results obtained by De Graef clarify a number of findings by earlier workers. Thus, patients with pernicious anaemia or atrophic gastritis were found to show a moderate elevation of the mucoproteose fraction, but a distinct diminution or absence of the mucoprotein fraction [118]. Similarly, rats in which glandular atrophy was induced by explantation of the stomach on to the abdominal/

al wall produced an alkaline fluid similar in composition to interstitial fluid, and containing mucoproteose, but never any acid, pepsin, or mucoprotein [119].

Origin of the Non-Parietal Component

If, as all the evidence seems to suggest, the non-parietal component is in effect a gastric interstitial fluid component, it is essential to enquire into its mode of formation, that is, into the determinants of transcapillary filtration in the vascular bed of the stomach.

A voluminous and often contradictory literature, ably reviewed by Jacobson [120], has accumulated on the circulation in the stomach. The effect of nervous factors and pharmacological agents has been investigated repeatedly on one or other of the circulatory parameters; regional blood flow, overall resistance, and mucosal blood content. Despite some contradictory observations, probably due to the widely different levels of doses used, there seems to be general agreement that gastrin, histamine and cholinergic agents, which act both as secretory stimulants and local vasodilators, as well as direct vagal stimulation, increase gastric mucosal blood flow and content, and decrease overall resistance [120, 121]. An opposite effect is obtained following administration of vasoconstrictive agents, such as noradrenaline and vasopressin [121, 122].

The early work of Beznack [123], and Koniges and Otto [124], had demonstrated by direct measurement that acetylcholine also increased/

increased splanchnic mucosal capillary pressure and promoted capillary filtration, but not until recently, and especially since the work of Folkow and his colleagues, have techniques been developed for continuous simultaneous recording of all circulatory events, and an attempt made at defining their relationship to capillary filtration. It will be recalled in this context that the main determinants of transcapillary filtration according to the Starling hypothesis are the mean effective capillary pressure (hydrostatic minus oncoctic) and the total capillary surface area available for filtration. The activity of pre-capillary sphincters affects the pattern of capillary flow as well as average capillary pressure and capillary surface area [125]. Blood flow and capillary filtration, however, may vary independently, even when the activity of submucosal shunts is discounted. Moreover, for similar blood flow rates, the amount of fluid filtered varies with the vascular bed. In this respect, the splanchnic circulation is uniquely endowed, with a capillary filtration rate at maximal vasodilatation analogous to glomerular filtration rates [126].

The continuous infusion of vasodilatory agents increases both splanchnic flow and capillary filtration [126]. Vasoconstrictor fibre stimulation, on the other hand, is accompanied by a decrease in filtration rate, which persists, though not to the same extent, despite the action of local vasodilatory mechanisms [127, 128, 129]. In the presence of unaltered blood flow and mean capillary pressure, this indicates a diversion of the blood away from the mucosal capillaries, and probably through submucosal arteriovenous shunts.

It/

It is in the context of transcapillary filtration studies that the movement of sodium as the index of the interstitial fluid component of juice should be interpreted. Folkow et al.[126] have shown that the increase in capillary blood flow and filtration was proportionate to the infused dose of vasodilator. The results presented in sections I and II show that the output of sodium in the first twenty minutes following infusion or single intravenous injections of gastrin II is also dose-dependent. The subsequent fall in sodium output may reflect local circulatory adjustments, and in the case of single intravenous injections the progressive disappearance of the stimulant from the tissues. Local adjustments affecting capillary blood flow, and to a lesser extent capillary filtration, have been found to occur following stimulation of vasoconstrictor fibres in animals[126, 130]. The possibility of the converse effect occurring following vasodilatation can theoretically be entertained and may provide an explanation for the return of the sodium output to basal levels during steady-states following infusion of gastrin II.

It was noted earlier that concomitant cholinergic stimulation increases the output of sodium of both histamine- and gastrin-stimulated juice, with a resultant increase in total volume and reduction in acidity. Atropine has the opposite effect[92]. The induction of vasoconstriction in animals by administration of pituitrin, barium salts, or by cooling of the stomach[122], results in a drastic reduction of secretion, while the acidity is maintained at high levels. Disproportionate increase or decrease, depending

on the agent used, in the rate of formation of interstitial fluid and its consequent passage into the gastric lumen, offers an adequate explanation of these phenomena.

Subcutaneous administration of large doses of gastrin appears to result in a larger output of sodium than does histamine. This may reflect the local release of histamine by gastrin inducing local circulatory changes without the concomitant lowering of systemic pressure induced by large doses of histamine and antihistamine [131]. Indeed, as has been noted by Haddy, the increase in capillary pressure and consequent transcapillary filtration is most evident following localised injection of histamine [132].

Transmucosal Passage of Interstitial Fluid.

Durbin et al. [133] have shown that in the chambered preparation of stripped frog gastric mucosa water moves along osmotic gradients, and by far the larger proportion of this water movement is accounted for by bulk flow rather than by diffusion. In the chambered preparation of canine gastric mucosa with intact blood supply, a similar movement of water and ions can also be demonstrated [112]. A hydrostatic gradient is even more effective in inducing filtration across the gastric mucosa than an equal osmotic gradient [134]. In the experiments of Altamirano [108], the movement of interstitial fluid into the lumen of the stomach following gastric intra-arterial infusion of acetylcholine could be quantitatively graded or totally blocked by applying hydrostatic pressure to the luminal surface of

the mucosa. The increased mucosal capillary pressure following acetylcholine, in conformity with the complementary findings of Folkow et al. [126], could attain levels similar to those found in glomerular capillaries, so that the movement of interstitial fluid out of the gastric capillary and its consequent passage into the lumen of the stomach is potentially very large.

Although calculated to account for around 15% of tissue volume, the width of the interstitial fluid compartment probably does not exceed 0.5 to 1 u. [135]. It is difficult to obtain reliable data on the pressure attained in the interstitial fluid compartment during filtration, but it would be safe to predict that it is proportionate to the rate at which interstitial fluid is formed. The gastric interstitial fluid compartment is interposed between plasma and a somewhat hyperosmotic primary acid secretion flowing over the mucosal surface. The combined osmotic effect of parietal secretion and hydrostatic effect in the tissue is probably responsible for the movement of interstitial fluid across the gastric mucosa. The locus of its passage is probably the intercellular interstices [136]. The architecture of the gastric mucous epithelium in man, as revealed by electron microscopy, with clear spaces at the base of the cells, appears well suited for this purpose [137].

Shortly after stimulation, the proportion of the interstitial fluid or non-parietal component may equal or exceed that of the parietal component, resulting in the low observed acidity. During maximal steady-state secretion following continuous infusion of gastrin in man, the proportion of non-parietal to parietal component

is/

is around 1:10 to 1:15. Following administration of large doses of histamine, when the acidity reaches its highest levels, the proportion may reach 1:30 to 1:40.

Damage to the mucosa in disease or experimentally by topical applications of eugenol may induce an abnormal passage of interstitial fluid [107]. This is in conformity with the frequent finding that inflammatory conditions of the stomach are accompanied by an increased volume of juice of low acidity and high protein content.

Concluding Remarks.

It was shown in the previous sections that an identical formulation to account for the relationship of acidity and secretory rate can be arrived at on the basis of the back-diffusion and two-component hypothesis. It is difficult, however, to fit the secretory data on sodium, obtained in this and other studies, to a simple diffusion equation. If account is taken of the bulk movement of interstitial fluid, and therefore of sodium, as it occurs in vivo, a quantitative description could be proposed that would eliminate any basic differences in the two models.

Section IV

The Two-Component Hypothesis of Pancreatic Secretion.

As a corollary to the findings of this study, a similar quantitative treatment can be shown to apply to pancreatic secretion. By analogy with gastric secretion, pancreatic secretion may be considered as a mixture in various proportions of a pancreatic bicarbonate component and an interstitial fluid component containing bicarbonate and chloride [138].

B = primary bicarbonate concentration in the pancreatic component.

b and Cl_i = primary bicarbonate and chloride concentrations in the interstitial fluid component.

k = volume of interstitial fluid component per sample.

v = observed secretory rate.

v - k = volume of primary pancreatic secretion.

All the primary concentrations, B, b and Cl_i are constant

(a) Bicarbonate.

The output of bicarbonate per sample may be expressed as follows:-

$$[HCO_3]\,v = B(v - k) + kb \qquad \text{Eq. h.1}$$

$$= Bv - k(B - b) \qquad \text{Eq. h.2}$$

$$[HCO_3] = B - k/v\,(B - b) \qquad \text{Eq. h.3}$$

$$k/v = \frac{B - [HCO_3]}{B - b} \qquad \text{Eq. h.4}$$

From/

From eq. h.3, it can be concluded that $[HCO_3]$ depends on the ratio of k/v, i.e. on the ratio of the interstitial fluid component to secretory rate.

In the presence of a constant interstitial fluid component (k now a constant), (i) the relationship $[HCO_3]$ and secretory rate, v, is hyperbolic, while that of $[HCO_3]$ and 1/v is linear;

(ii) the relationship of bicarbonate output and secretory rate is linear (eq. h.2).

At infinitely high secretory rates, $1/v = 0$ and $[HCO_3] = B$

At $v = k$, $[HCO_3] = b$

(b) Chloride.

The chloride originates in the interstitial fluid component and its output per sample may be expressed as follows:-

$$[Cl]\ v = Cl_i k \qquad \text{Eq. i.1}$$

$$[Cl] = \frac{Cl_i k}{v} \qquad \text{Eq. i.2}$$

$$k/v = [Cl]/\ Cl_i \qquad \text{Eq. i.3}$$

From Eq. i.2, it can be concluded that $[Cl]$ is dependent on the ratio of k/v. In the presence of a constant interstitial fluid component, the relationship of $[Cl]$ and secretory rate is hyperbolic, while that of $[Cl]$ and 1/v is linear.

At $1/v = 0$, $[Cl] = 0$

At $v = k$, $[Cl] = Cl_i$

(c) /

(c) Relationship of the concentrations of chloride and bicarbonate.

Combining Eqs. h.4 and i.3,

$$[Cl] = \frac{Cl_i\ (\ B - [HCO_3])}{B - b} \qquad \text{Eq. j.1}$$

from which it can be concluded that the relationship of $[Cl]$ and $[HCO_3]$ is linear.

At $[Cl] = 0$, $[HCO_3] = B$, the primary pancreatic bicarbonate.

At $[Cl] = Cl_i$, $[HCO_3] = b$, the interstitial fluid bicarbonate.

These theoretical conclusions were put to the test on published human (Dreiling and Janowitz, 1956) [139], and canine (Hart and Thomas, 1945[140], Christodoulopoulos et al., 1961 [141]) pancreatic electrolyte data.

(i) The linear relationship of $[HCO_3]$ and $[Cl]$ is evident in both canine and human data, (Fig. 15). All the points lie on the same regression line, indicating that the primary concentrations in both components are similar in the two species.

(ii) The index of the interstitial fluid component in the context of pancreatic secretion is the chloride output. If the data derived from human subjects [139] are grouped in series made up of/

of samples with similar chloride outputs, so that the value of k is a constant, the predicted linear relationship of $[HCO_3]$ and 1/v becomes evident (Fig. 16). It should be noted that this transformation was necessitated by the fact that these data were obtained during non-steady-state secretion induced by single intravenous injections of secretin.

(iii) Closer scrutiny of human and canine data shows that the chloride output, like its counterpart the sodium output of gastric juice, rises in the first 10 to 20 minutes and is dose-dependent[141].

(iv) The primary concentrations are independent of the nature of the stimulant or mode of stimulation. Thus the human data in Fig. 15 were obtained following stimulation by intravenous secretin, while the canine data were obtained by various means, including injection of secretin extracts and irrigation by peptone, soap, or hydrochloric acid.

(v) It can be shown by adding the factor of $[HCO_3]$ to both sides of Eq. j.1 that the sum of $[HCO_3]$ and $[Cl]$ is not constant, but rises to a maximum equal to B and falls to a minimum equal to $Cl_i + b$, the sum of the bicarbonate and chloride concentrations in the interstitial fluid component. The slope of the regression line relating $[HCO_3] + [Cl]$ to $[HCO_3]$ is small, so that the full significance of this observation can only be judged in the presence of an appreciable span of data (Fig. 15).

(vi) Separate regression analysis of $[Cl]$ on $[HCO_3]$ shows closely similar intercepts for human and canine data. If a value

is attributed to b analogous to its value in plasma or interstitial fluid, i.e. 25 mEq/L, the concentration of Cl_i can be obtained.

A summary of the results is presented in Table VI.

It is of interest to note the similar concentration levels of the primary pancreatic bicarbonate and primary gastric parietal chloride in both species. As predicted, the calculated value of Cl_i is fairly close to its expected value in interstitial fluid.

(vii) Comparison of the canine and human data shows that the lower $[HCO_3]$ in the latter is due to the higher proportion of interstitial fluid, as indicated by the larger content of chloride in the human specimens [139].

The highest $[HCO_3]$ observed in dogs was around 160 mEq/L. It appears from Fig. 16 that pure pancreatic secretion does not contain chloride ($[Cl] = 0$ at $1/v = 0$). It can, however, be shown, by extending Eqs. h.1 - h.4, that even if primary pancreatic secretion contained chloride, the linear relationship of $[HCO_3]$ and $[Cl]$ persists, and only the value attributed to the primary bicarbonate, B, changes.

(viii) The administration of anticholinergic agents leads to a decrease in pancreatic secretory rate accompanied by a rise in $[HCO_3]$ [142]. This is probably attributable by analogy with gastric secretion to their disproportionate effect on the interstitial fluid component.

Similarly, the proportion of the interstitial fluid component, as indicated by the chloride output, is larger in patients with pancreatitis/

pancreatitis than in normal subjects, and the observed $[HCO_3]$ is lower. This situation is analogous to that encountered in gastritis. The relationship of $[HCO_3]$ and $[Cl]$, which is independent of k/v, the volume factor, remains undisturbed (Fig. 15).

The parallelism between gastric and pancreatic secretion in the development of the equations, the fit of the data, and the conclusions (i to viii) is clearly evident.

The formulation of a two-component hypothesis to account for the relationship of electrolyte concentration and secretory rate is not unique to gastric secretion. It would appear that the observed variations in electrolyte concentration in the secretions of these two organs are not so much the product of a specific regulatory mechanism as a concomitant of the normal outward passage of interstitial fluid.

	n	r	Primary Bicarbonate B	Primary Chloride Cl_1
MAN	21	- 0.97	166.0 ± 5.5	124.4 ± 1.2
DOG	54	- 0.97	167.2 ± 1.5	123.8 ± 3.1
COMBINED	75	- 0.99	168.3 ± 1.1	124.7 ± 1.3

TABLE VI. Calculated primary concentrations in pancreatic juice.

n: number of samples

r: coefficient of correlation.

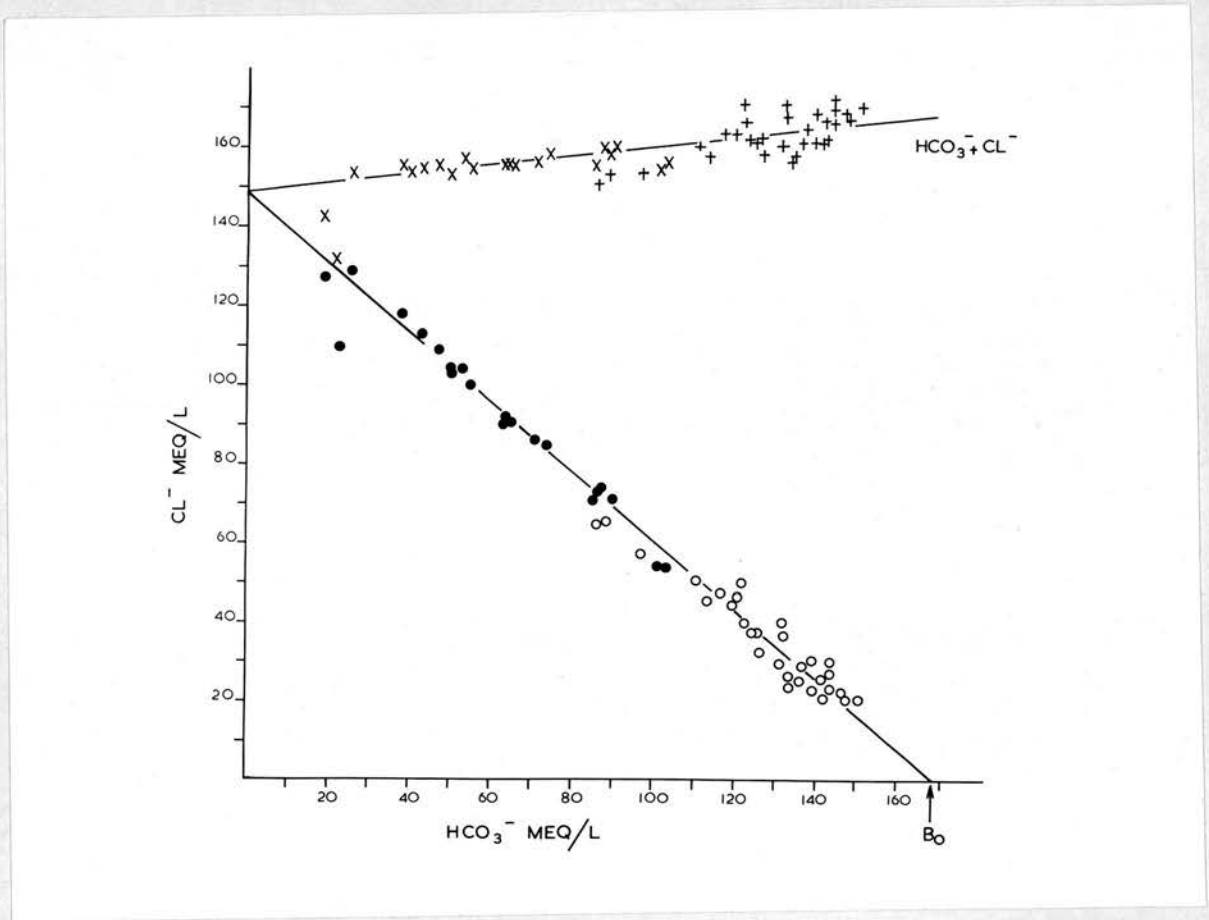


Figure 15: The linear relationship of bicarbonate and chloride concentrations in pancreatic secretion. The closed circles and X represent data obtained from man; the open circles and crosses represent data from dogs.

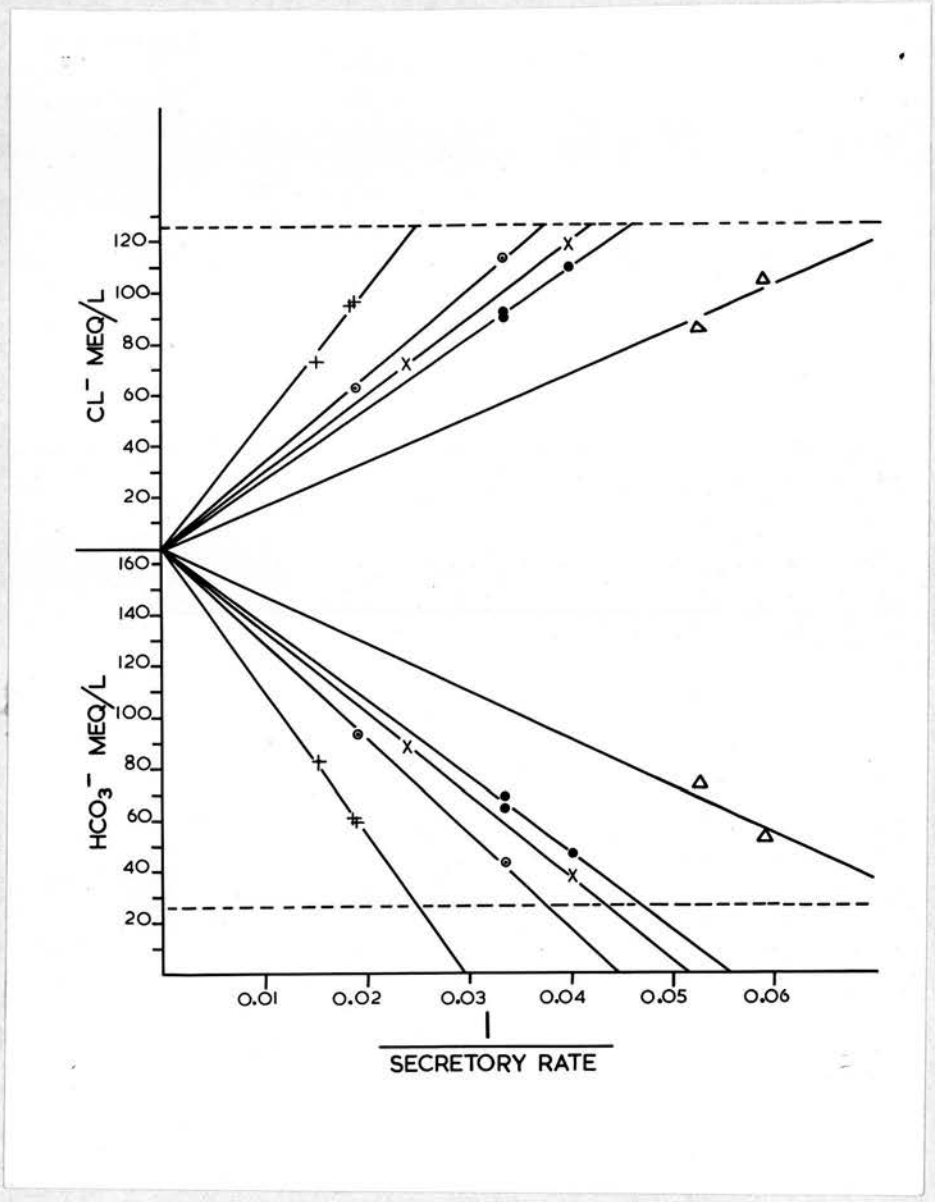


Figure 16: The linear relationship of bicarbonate and chloride concentration to 1/v in mm. The dotted lines represent the calculated limits of the interstitial fluid component. Each series is made up of samples with similar chloride outputs.

Note on the Gastric Electrolytes in Patients with Pernicious Anaemia

Two patients were tested with 2 ug/kg of gastrin. Both responded by producing an alkaline fluid containing measurable amounts of bicarbonate (range of concentration: 5.8 to 15.8 mEq/L).

The electrolyte concentration pattern towards the latter half of the test was closely similar to the concentration pattern in antral fluid (Figs. 1 and 2). The latter is distinguished by sodium and potassium levels slightly higher than the corresponding interstitial fluid levels. The chloride and bicarbonate concentrations vary in a reciprocal manner and their sum is virtually identical with that in interstitial fluid. It is possible that antral fluid does not represent an active secretion but simply an interstitial fluid subjected to a chloride-bicarbonate shift after passage into the antral lumen.

Mrs. B. - Pernicious Anaemia. (Gastrin 2 ug/kg.)

Secretory Rate/10'	pH	Na^+	K^+	Na^+K	HCo_3^-	Cl^-	$Cl^-+HCo_3^-$
17.00	6.90	128.00	11.25	139.25	5.85	127.50	133.30
10.60	7.00	123.50	10.50	134.00	6.61	124.50	131.10
13.40	7.20	133.00	8.00	141.00	6.35	129.50	135.85
8.30	7.32	134.50	8.50	143.00	6.81	131.50	138.30
9.00	7.45	140.00	7.00	147.00	9.63	132.50	142.10
6.20	7.50	139.00	7.75	146.75	10.35	130.00	140.30
4.00	7.40	140.50	7.75	148.25	7.99	134.50	142.50

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